Fate of herbicides in deep subsurface limestone and sandy aquifers

Janniche, Gry Sander

Publication date:
2010

Document Version
Publisher's PDF, also known as Version of record

Technical University of Denmark

Fate of herbicides in deep subsurface limestone and sandy aquifers

Gry Sander Janniche

DTU Environment
Department of Environmental Engineering

PhD Thesis
April 2010

Fate of herbicides in deep subsurface limestone and sandy aquifers

Gry Sander Janniche

PhD Thesis
April 2010

DTU Environment
Department of Environmental Engineering
Technical University of Denmark

Gry Sander Janniche

Fate of herbicides in deep subsurface limestone and sandy aquifers

PhD Thesis, April 2010

The thesis will be available as a pdf-file for downloading from the homepage of the department: www.env.dtu.dk

Address:	DTU Environment Department of Environmental Engineering Technical University of Denmark Miljoevej, building 113 DK-2800 Kgs. Lyngby Denmark
Phone reception:	+45 4525 1600
Phone library:	+45 4525 1610
Fax:	+45 4593 2850
Homepage:	http://www.env.dtu.dk
E-mail:	reception@env.dtu.dk

Printed by:	Vester Kopi Virum, April 2010
Cover:	Torben Dolin
ISBN:	978-87-91855-90-0

Preface

This PhD thesis is based on work carried out at the Department of Environmental Engineering at the Technical University of Denmark during January 2005 to March 2010 with Professor Hans-Jørgen Albrecthsen as supervisor. The PhD project was funded partly by DTU and partly by the European Union FP6 Integrated Project AquaTerra (Project no. GOCE 505428) under the thematic priority: sustainable development, global change and ecosystems.

This PhD thesis is composed of a summary of herbicide sorption and degradability in subsurface limestone and sandy aquifers with specific focus on the herbicides mecoprop, isoproturon, atrazine and acetochlor, as well as the following four papers which have been submitted to international peer-reviewed journals.

I. Janniche, G., Lindberg, E., Mouvet, C., Albrechtsen, H.-J. Distribution of isoproturon, mecoprop and acetochlor mineralization and mineralizers in deep unsaturated limestone and sandy aquifer, submitted to *Chemosphere*.

II. Janniche, G., Mouvet, C., Albrechtsen, H.-J. Vertical small-scale variations of sorption and mineralization of three herbicides in subsurface limestone and sandy aquifer, submitted to *Journal of Contaminant Hydrology*.

III. Janniche, G., Albrechtsen, H.-J. Acetochlor sorption and mineralization in limestone subsurface and aquifers, submitted to *Science of the Total Environment*.

IV. Janniche, G., Spliid, H., Albrechtsen, H.-J. Application of microbial Community-Level Physiological Profiles and herbicide mineralization to a herbicide contaminated groundwater, submitted to *FEMS Microbiology Ecology*.

They are in the thesis referred to with the roman numerals accentuated in bold (e.g. Janniche et al. **I)**, because one main purpose of the summary was to compare them with the available literature.
The papers are not included in this www-version, but can be obtained from the Library at DTU Environment: Department of Environmental Engineering, Technical University of Denmark, Miljoevej, Building 113,
DK-2000 Kgs. Lyngby, Denmark, library@env.dtu.dk.

During my PhD study I have cooperated with external partners and participated in project meetings because my study to a great extent was financed by the EU project AquaTerra. Furthermore, I have been in France to collect samples from a limestone catchment. The sampling was done in collaboration with Dr. Christophe Mouvet from BRGM (Bureau de Recherches Géologiques et Minières) whom I have been in continuous contact with. In this way I have achieved acquaintance of other working cultures and participated in knowledge sharing on several levels. Furthermore, I was in charge of reporting my research to the coordinator of the EU project by writing Deliverables in collaboration with other partners (primarily BRGM and TNO). I have also presented my research at national and international conferences with both posters and oral presentations. This has lead to the following 7 conference proceedings:

Danish conference proceedings:

Janniche, G.S.; Albrechtsen, H.-J. Acetochlors skæbne fra topjord, gennem kalk til grundvandsmagasin: Undersøgelser af et nyt herbicid. Vintermøde om jord- og grundvandsforurening, Vingstedcentret 10.-11. marts 2009; Bind 1, p. 131-138. Kgs. Lyngby, ATV Jord og Grundvand, 2009.

Janniche, G.S.; Lindberg, E.; Albrechtsen, H.-J. Småskala-variation i nedbrydning af pesticider (acetochlor, isoproturon og MCPP). Vintermøde om jord- og grundvandsforurening, Vingstedcentret 6.-7. marts 2007; Bind 2, p. 215-225. Kgs. Lyngby, ATV Jord og Grundvand, 2007.

International conference proceedings:

Janniche, G.S.; Baran, N.; Gutierrez, A.; Mouvet, C.; Albrechtsen, H.-J. Fate of herbicides in Brévilles catchment from topsoil through aquifer to spring. AquaTerra; Final conference - Processes - data - models - future scenarios. Scientific fundamentals for river basin management, 25th to 27th March 2009; Tübingen, Germany; Conference programme & proceedings, p. 23; Tübingen: Institut für Geowissenschaften der Universität Tübingen, 2009.

Albrechtsen, H.-J.; **Janniche, G.S.**; Lindberg, I.E.; Mouvet, C. Microbial degradation of pesticides in subsurface: Scale matters. 7th International Symposium on Subsurface microbiology (ISSM 2008), Shizuoka, Japan, 16-21 November 2008; Abstract Book, p. 20; Kimura, H., Shizuoka, Japan: Shizuoka University, 2008.

Janniche, G.S.; Lindberg, I.E.; Mouvet, C.; Albrechtsen, H.-J. Spatial small-scale variation in degradation of pesticides (acetochlor, isoproturon MCPP) in limestone and sand from Brévilles, France. International workshop on "Biogeochemical processes in groundwater systems", GSF Campus, Munich, Germany 10-11 September 2007; Neuherberg: GSF - National Research Center for Environmental and Health in the Helmholtz Association, 2007.

Janniche, G.S.; Lindberg, E.; Mouvet, C.; Albrechtsen, H.-J. Small-scale variation in degradation of pesticides (acetochlor, isoproturon and MCPP) in unsaturated and aquifer environments. AquaTerra Subproject Meeting BIOGEOCHEMC Combined with KNOWMAN course on Brévilles: Long-term fate of pollutants in soils: Mobility, stability, and transformation 15-16 March, 2007, p. 15-Tübingen: Eberhard Karls Universität Tübingen, 2007.

Janniche, G.S.; Clausen, L.; Nygaard, B.; Albrechtsen, H.-J. Degradation and sorption in subsurface and aquifers of the herbicide metabolite BAM after non-point contamination. Pesticide behaviour in soils, water and air: Oral and poster abstracts, University of Warwick, UK, 27-29 March 2006-London, UK: SCI Pest Management Group, 2006.

Furthermore, I am co-author on the following article:

Barth, J.A.C.; Steidle, D.; Kuntz, D.; Gocht, T.; Mouvet, C.; von Tümpling, W.; Lobe, I.; Langenhoff, A.; Albrechtsen, H.-J.; **Janniche, G.S.**; Morasch, B.; Hunkeler, D.; Grathwohl, P. Deposition, persistence and turnover of pollutants: first results from the EU project AquaTerra for selected river basins and aquifers. *Science of the Total Environment*, vol: 376, p. 40-50 (2007).

Acknowledgements

There are many people I wish to thank: My supervisor Hans-Jørgen Albrechtsen for giving me the opportunity to do a PhD in this very interesting field and also for always being willing to read and discuss my work, Christophe Mouvet, Nicole Baran, and Alexis Gutierrez, all from BRGM, not only for your good cooperation during all these years, but also for your critical review of manuscripts. My thanks also goes to Anitha Sharma for her constructive comments on my thesis. A special thank you goes to Henrik Spliid for all the time you spent on me and the EcoPlate results performing statistics. Furthermore, I wish to thank Mona Refstrup, Birthe Ebert, Sinh Nygen, Susanne Kruse and all the other lab technicians at DTU Environment, as well as Torben Dolin for the professional drawings of my graphs, for your work as well as your support which were greatly appreciated. It has been a pleasure working at DTU Environment so a thank you goes to all my enthusiastic colleagues.

Finally, I wish to thank my wonderful family and friends for your support and for having patience with me – especially the last couple of months of this PhD study. My gratitude goes to my very dear husband Thomas and my two fantastic daughters, Mathilde and Nikoline, for always being there for me.

March 2010
Gry Sander Janniche

Abstract

Use of herbicides may cause them to leach from the topsoil to the vadose zone and further into the groundwater. In many countries, drinking water is extracted from groundwater, and thus herbicides in groundwater pose a threat to the quality of the drinking water. Knowledge on sorption and degradation is essential in order to assess the herbicidal fate in subsurface and aquifers. This has also to a wide extent been investigated in shallow environments, but rarely in sediments from depth below 10 m below surface (mbs) and at environmentally relevant low concentrations (<10 µg/L). The **aim** of this PhD thesis was to study sorption and degradation in limestone and sandy aquifers from >10 mbs at low concentrations of four different herbicides (atrazine, acetochlor, mecoprop, and isoproturon). A secondary aim was to study spatial small-scale variation in sorption and degradation in relation to evaluating fate of herbicides.

The four herbicides studied belong to different groups of herbicides with different chemical properties, and they are among the most used herbicides worldwide. Atrazine was banned in 2004 in EU, and the relatively new herbicide acetochlor has to a wide extent substituted atrazine. Acetochlor sorption and degradation has not previously been studied in limestone and aquifers even though it was studied in topsoil and down to 5 mbs. Neither have sorption and degradation been studied previously in these two environments in >10 mbs at <10 µg/L. The literature review therefore included studies at both higher concentrations and shallower depths. Furthermore, chalk was included as only few studies have been performed previously with limestone.

Sorption to **limestone** and chalk increased in the order mecoprop<atrazine< acetochlor<isoproturon, but was in general limited; the highest linear distribution coefficient (K_d) was 0.55 L/kg. Thus, these geological settings provide only limited protection from groundwater pollution. The herbicides will, however, remain bioavailable. Atrazine was recalcitrant in limestone and chalk, but was still *degraded* in both shallow and deep samples at an initial concentration of >5mg/L, which is extremely high even for a point source pollution. Acetochlor was degradable, and mineralized with half-lives of more than 4 years at about 100 µg/L (10 µg/kg). The half-lives were substantially longer at low initial concentrations (>27 year at 7 µg/L corresponding to 1 µg/kg) than at high, while there was no considerable difference between half-lives of shallow and deep

samples. Mecoprop was also mineralized with half-lives from 1 year in shallow samples and from 9.5 years in deep samples (<10 µg/L). Isoproturon was in some samples degraded fast without being mineralized, and in other samples isoproturon was mineralized with half-lives of >2 years in shallow samples and >95 years in deep samples (<10 µg/L). Hence, acetochlor, mecoprop, and isoproturon may be removed completely (mineralized) in >10 mbs at environmentally relevant low concentrations, though slowly.

Sorption to **sandy aquifers** increased in the order mecoprop<atrazine< isoproturon<acetochlor, and sorption was highest in anaerobic sediments. Mecoprop sorption was limited and at the same level as in limestone and chalk, while isoproturon and acetochlor sorption was considerable; up to 5.12 L/kg. Hence, retardation due to sorption can be substantial for isoproturon and acetochlor in sandy aquifers. Atrazine was *degraded*, but not mineralized in shallow and deep sandy aquifers, but only at an initial concentration of 4.5 mg/L. Yet, mineralization at 1 µg/L was observed in one out of 13 deep groundwater samples (without sediment) from a sandy aquifer. 1 µg/L acetochlor was only mineralized in few deep samples with half-lives of 9.5-32 years. Mecoprop was degraded and mineralized in almost every sample, and for deep samples at low concentrations the half-lives were 1.0-9.5 year. Though the half-lives were long especially for isoproturon and acetochlor this slow mineralization can be significant considering the long residence time in the aquifer system.

Though the herbicides acetochlor, mecoprop, and isoproturon were degradable in limestone/chalk and sandy aquifers, they were not degraded in every sample. There is therefore a spatial variation in the degradation potential. Variation in both sorption and degradation can even be large within few cm distances; **small-scale variation**. For example, the acetochlor sorption increased with depth approximately 78 % pr 5 cm in a 33 cm long core of sandy aquifer, and in a 50 cm long limestone core the isoproturon and acetochlor sorption was 4-5 times higher, plus more than five times more mineralization in the top 30 cm compared to the deepest 20 cm. The importance of these small-scale variations for herbicide fate depend on the magnitude of these layers with increased degradation and sorption, and inclusion of these in model calculations of herbicide fate may improve the predictions of groundwater pollution.

It is **concluded** for environmentally relevant low concentrations (<10 µg/L) in samples deeper than 10 mbs: 1) that *limestone/chalk* provides only limited protection against groundwater pollution with mecoprop, atrazine, isoproturon, and acetochlor, because the sorption is low, and the mineralization very slow for mecoprop, isoproturon, and acetochlor, and because atrazine is not degradable; 2) that in *sandy aquifers* sorption of the four herbicides is in general low, but can be substantially for especially isoproturon and acetochlor under reduced conditions. Mecoprop, isoproturon, and acetochlor, but not atrazine may furthermore be degraded and even mineralized slowly; 3) that *small-scale variations* in sorption and degradation were considerable even within few cm horizontal distance. The importance of this variation depends on the total magnitude of the layers with increased sorption or degradation.

Dansk resumé

Brug af herbicider kan medfører at de udvaskes fra topjorden til den umættede zone og videre til grundvand. Drikkevand indvindes i mange lande fra grundvand og dermed udgør herbicider i grundvand en trussel mod drikkevandskvaliteten. Viden om sorption og nedbrydning er essentiel for at vurdere herbiciders skæbne i underjord og grundvandsmagasiner. Dette er da også undersøgt i vid ustrækning i overfladenære miljøer, men sjældent i sedimenter dybere end 10 m under terræn (mut) og ved miljø-relevante lave koncentrationer (< 10 µg/L). **Formålet** med denne PhD afhandling var at undersøge sorption og nedbrydning i kalksten og sandede grundvandsmagasiner fra >10 mut ved lave koncentrationer af fire forskellige herbicider (atrazin, acetochlor, mecoprop og isoproturon). Et sekundært formål var at undersøge rumlig småskala-variation i sorption og nedbrydning i forhold til at evaluere herbicidernes skæbne.

De fire undersøgte herbicider tilhører hver sin gruppe af herbicider med forskellige kemiske egenskaber, og er nogen af de mest brugte herbicider på verdensplan. Atrazin blev i 2004 forbudt i EU, og det forholdsvis nye herbicid acetochlor har i høj grad substitueret atrazin. Selvom acetochlor sorption og nedbrydning er undersøgt i topjord og ned til 5 mut, er det ikke tidligere undersøgt i kalksten eller i grundvandsmagasiner. I disse to miljøer er sorption og nedbrydning af de fire herbicider heller ikke tidligere blevet undersøgt i >10 mut ved <10 µg/L. Derfor blev der i litteraturstudiet medtaget undersøgelser ved både højere koncentrationer og lavere dybder. Der udover blev kalk også medtaget, da kun få studier er udført med kalksten.

Sorption til **kalksten** og kalk steg i følgende rækkefølge: mecoprop<atrazin< acetochlor<isoproturon, men var generelt begrænset; den højeste lineære distributionskoefficient (K_d) var 0,55 L/kg. Dermed yder sådanne geologiske lag kun ringe beskyttelse mod grundvandsforurening. Herbiciderne vil dog forblive biotilgængelige. Atrazin var svært *nedbrydeligt* i kalk og kalksten, men blev dog nedbrudt i både terrænnære og dybe prøver ved initialkoncentration på >5 mg/L, hvilket er ekstremt højt selv for en punktkildeforurening. Acetochlor var nedbrydeligt, og blev mineraliseret med halveringstider på mere end 4 år ved ca. 100 µg/L (10 µg/kg). Halveringstiderne var betydelig længere ved lave initialkoncentrationer (>27 år ved 7 µg/L svarende til 1 µg/kg) end ved høje, mens der ingen væsentlig forskel var på halveringstiderne for terrænnære og

dybe prøver. Mecoprop blev også mineraliseret med halveringstider fra 1 år i de terrænnære prøver og fra 9.5 år i de dybe prøver (<10 µg/L). Isoproturon blev i nogle prøver hurtigt nedbrudt uden at mineraliseres. I andre prøver blev isoproturon dog mineraliseret med halveringstider på >2 år i terrænnære prøver og >95 år i dybe prøver (<10 µg/L). Der kan således ske en fuldstændig fjernelse (mineralisering) af acetochlor, mecoprop og isoproturon i >10 mut ved miljømæssige relevante lave koncentrationer, om end den er langsom.

Sorption til **sandede grundvandssedimenter** steg i følgende rækkefølge: mecoprop<atrazin<isoproturon<acetochlor, og sorptionen var størst i anaerobe sedimenter. Mecoprop sorptionen var begrænset og på samme niveau som i kalk og kalksten, mens isoproturon og acetochlor sorptionen var betydelig; op til 5,12 L/kg. Retardationen pga. sorption kan således være betydelig for primært isoproturon og acetochlor i sandede grundvandsmagasiner. Atrazin blev *nedbrudt,* men ikke mineraliseret i sandede grundvandsmagasiner i både terrænnære og dybde prøver, men kun ved en initialkoncentration på 4.5 mg/L. Alligevel blev mineralisering ved 1 µg/L observeret i én ud af 13 dybe grundvandsprøver (uden sediment) fra et sandet grundvandsmagasin. 1 µg/L acetochlor blev kun mineraliseret i enkelte dybe prøver med halveringstider på 9.5-32 år. Mecoprop blev nedbrudt og mineraliseret i næsten alle prøver, og for dybe prøver med lave koncentrationer var mineraliseringshalveringstiderne 1,0-9,5 år. Selv om halveringstiderne for især isoprotuon og acetochlor var lange, kan denne langsomme mineralisering have betydning for stoffjernelsen, når blot opholdstiderne er tilsvarende lange.

Selv om herbiciderne acetochlor, mecoprop og isoproturon var nedbrydelige i kalk/kalksten og sandede grundvandsmagasiner, blev de ikke nedbrudt i alle prøver. Der er således en rumlig variation i nedbrydningspotentialet. Selv indenfor afstande på få cm kan der være stor variation i både sorption og nedbrydning; **småskala-variation**. Eksempelvis steg sorptionen af acetochlor over dybden i en 33 cm lang kerne fra sandet grundvandsmagasin med ca. 78 % pr. 5 cm, og en 50 cm lang kalkstenskerne havde 4-5 gange højere isoproturon og acetochlor sorption samt mere end 5 gange mere mineraliseret i de øverste 30 cm i forhold til de nederste 20 cm. Betydningen af disse småskala variationer for herbicidernes skæbne afhænger af udbredelsen af disse lag med høj nedbrydning og sorption, og medtages disse i modelberegninger af herbicidernes skæbne kan forudsigelser af risikoen for grundvandsforurening forbedres.

Det **konkluderes** ved miljø-relevante lave koncentrationer (<10 µg/L) for prøver dybere end 10 mut: 1) at *kalk/kalksten* yder ringe beskyttelse mod grundvands-forurening af mecoprop, atrazin, isoproturon og acetochlor, da sorptionen er lav og mineraliseringen meget langsom for isoproturon, acetochlor og mecoprop, og atrazin ikke er nedbrydeligt; 2) at i *sandede grundvandsmagasiner* er sorptionen af de fire herbicider general lav, men kan være kraftig for især isoproturon og acetochlor under reducerede forhold. Mecoprop, isoproturon og acetochlor, men ikke atrazin, kan desuden nedbrydes og endda mineraliseres langsomt; 3) at s*måskala-variation* i sorption og nedbrydning var tydelig selv med få cm horisontal afstand. Betydningen af denne variation afhænger af den samlede udbredelse af lag med forhøjet sorption eller nedbrydning.

Table of content

1 Introduction

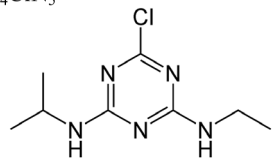
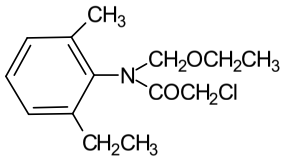
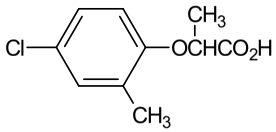
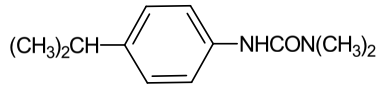
Herbicides have for several decades been used to a wide extent in agriculture, and their presence in the topsoil may be a threat to groundwater quality if they leach to groundwater. Groundwater often constitute important drinking water resources and hence the European Commission has set threshold limit values for pesticides and their metabolites in groundwater at 0.1 µg/L for each pesticide or metabolite and 0.5 µg/L for the sum of pesticides and metabolites (European Parliament and Council, 2006). Unfortunately, herbicides are often detected in groundwater, and often at concentrations above 0.1 µg/L (e.g. Baran et al., 2007; GEUS, 2009; Hallberg, 1989; Kalkhoff et al., 1998; Kolpin et al., 1998) posing a substantial risk to drinking water resources. This diffuse pesticide pollution has become one of the major environmental challenges for using groundwater as drinking water, and in order to predict the fate of pollutants in groundwater it is necessary to consider the entire catchment (Turner et al., 2006). Several important drinking water resources are located in chalk and limestone catchments e.g. in Denmark, France, and UK. The chalk or limestone may harbor the aquifer or may overlay the aquifer. In any case the groundwater in or below the limestone is or has been in contact with the limestone for often quite a long time; during the vertical leaching to the aquifer and/or subsequently during the storage or horizontal transport in the aquifer. This environment is distinctly different from topsoils with respect to e.g. content of nutrients, oxygen, water, carbon and clay which also may lead to very different microbial communities (Griebler and Lueders, 2009) and different degradation potential of herbicides.

One of the most often found herbicides in groundwater is atrazine (table 1) (Battaglin et al., 2000; Boyd, 2000; GEUS, 2007), used for control of weeds in maize since the 1960s in Europe. It was first in 2003 France banned atrazine after several findings in the groundwater, and it was afterwards banned in all EU countries (The Commission of the European Communities, 2004). The atrazine substitute acetochlor (table 1) was registered in France in 2000, and in 2003 it was the most used plant protection product in maize crops in France (Nadin, 2007). First detections of acetochlor in groundwater were in USA in 1995; only a year after its introduction on the US market (Kolpin et al., 1997). Since then several studies report its presence in groundwater (Battaglin et al., 2000; Boyd, 2000; de Guzman et al., 2005; Kalkhoff et al., 1998; Kolpin et al., 1998). Isoproturon and mecoprop (table 1) are herbicides often used in cereals and are

also frequently detected in groundwater (GEUS, 2009; Scheidleder J and et al., 1999; Spliid and Koppen, 1998). Mecoprop was in 2003 among the most used herbicide in cereal in several EU countries, and isoproturon was the second most extensively used herbicide in agriculture in Europe in 2003 with 12073 tonnes active substance which is equivalent to 14% of the total mass of herbicide used in EU (Nadin, 2007).

Knowledge on sorption and degradation is essential for evaluating risks of herbicide contamination of groundwater since these two processes are the major drivers in Natural Attenuation. The fate of herbicides in the subsurface depends to a high degree on the ability of the microbial population to degrade the herbicides – ideally by complete mineralization of the parent compound into CO_2. Degradation of herbicides is often considered to decrease with depth (Albrechtsen et al., 2001; Fomsgaard, 1995; Larsen et al., 2000; Wood et al., 2002), but degradation can be faster in sub-soil than in top-soil (Mills et al., 2001). Sorption may also decrease with depth because of decreasing organic matter content which on the other hand may increase the bioavailability (Bending and Rodriguez-Cruz, 2007). Degradation studies of herbicides in topsoil and shallow subsurface are numerous (e.g. Bending and Rodriguez-Cruz, 2007; Buss et al., 2006; Issa and Wood, 2005; Sorensen et al., 2003; Walker et al., 2001; Wood et al., 2002) likewise in shallow aquifers (e.g. Agertved et al., 1992; Albrechtsen et al., 2001; Larsen et al., 2000; Torang et al., 2003; Tuxen et al., 2002). Only few studies have considered the degradation of herbicides in chalk or limestone (Besien et al., 2000; Chilton et al., 2005; Johnson et al., 1998; 2000; 2003; Mouvet et al., 2004) despite the importance of this environment as water resource. However, the complexity and heterogeneity of this environment and the challenges in drilling and sampling these settings often being hard rock may be the reason for the few investigations. The challenges in sampling deep (>10 m below surface (mbs)) aquifers may also be the reason why only few investigations have considered this environment when investigating the fate of herbicides, despite deep aquifers are important as drinking water resources – and in many cases are contaminated by herbicides (GEUS, 2009; Rugge et al., 2005).

Table 1: Herbicide information (based on Tomlin, 1997)

	Atrazine	Acetochlor	Mecoprop	Isoproturon
Group	1,3,5-triazine	Chloroacetanilide	Aryloxyalkanoic acid	Urea
IUPAC name	6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine	2-chloro-*N*-ethoxymethyl-6'-ethylaceto-*o*-toluidide	(RS)-2-(4-chloro-*o*-tolyloxy)propanic acid	3-(4-Isopropylphenyl)-1,1-dimethylurea
Formula	$C_8H_{14}ClN_5$	$C_{14}H_{20}ClNO_2$	$C_{10}H_{11}ClO_3$	$C_{12}H_{18}N_2O$
Structure				
Molecular weight (g/mol)	215.7	269.8	214.6	206.3
Solubility in water, 25 ˚C (mg/L)	33 (22 ˚C)	233	734	65 (22 ˚C)
Log K_{ow}, 25 ˚C	2.5	3.03	0.10	2.5 (20 ˚C)
pK_a	1.7	N-I	3.78	N-I
WHO toxicity class	Slightly hazardous	Slightly hazardous	Slightly hazardous	Slightly hazardous
Use	Pre- and post-emergence control of annual broad-leaved weeds and annual grasses in e.g. maize and sorghum	Pre-emergence or pre-plant control of annual grasses, certain annual broad-leaved weeds and yellow nutsedge in e.g. maize	Post-emergence control of broad-leaved weeds in e.g. wheat and barley	Pre- and post-emergence control of annual grasses and many annual broad-leaved weeds in e.g. wheat and barley
Use in EU	Banned in 2004	Voluntarily withdrawn by 12/2010 (Never approved in DK)	Yes (restricted use in DK)	Yes (Banned in DK)

IUPAC: International Union of Pure and Applied Chemistry. N-I: Non-ionized.

1.1 Aim and approaches

The aim of this PhD thesis was to study natural sorption and degradation at low concentrations (µg-range) of the herbicides mecoprop, isoproturon, acetochlor and atrazine in deep (>10 mbs) subsurface samples of limestone, sandy aquifer and groundwater. A secondary aim was to investigate spatial small-scale variation in sorption and degradation in relation to evaluation of herbicide fate. The **novelty** of this PhD study can be summarized to studies performed at environmentally relevant low concentrations and in rarely studied environments:

- limestone from deep subsurface (<43 mbs)
- aquifer sediment from deep aquifer (10-59 mbs)
- furthermore, with acetochlor, a new herbicide in EU

The four specific herbicides were chosen because they were all heavily used worldwide, and because they represent four different groups of herbicides (table 1) with different chemical properties. Isoproturon and acetochlor are hydrophobic, non-polar organic herbicides whereas mecoprop and atrazine are hydrophilic (table 1). Mecoprop is a phenoxy acid with a pKa value of 3.78 and at near-neutral pH it is therefore negatively charged whereas atrazine is a week base behaving as a uncharged compound except under extreme acidic conditions (Clausen et al., 2001).

The results obtained during this PhD study are compared with literature from other studies of limestone when possible. However, work performed on limestone is often scarce and therefore results from chalk have been included. It is important to conduct degradation investigations at conditions that mimic in-situ as much as possible e.g. realistic concentrations and temperatures. My focus is on diffuse herbicide pollution, and not pollution due to point sources, and therefore on low initial herbicide concentrations (<5 µg/kg). I have only investigated biological degradation, and I distinguish between degradation and mineralization: degradation is the initial step where the herbicide is degraded to metabolites, whereas mineralization is complete degradation of the herbicide to CO_2 and inorganic ions.

2 Carbonate rock systems

Carbonate rock is a sedimentary rock characterized by a content of ≥85 % carbonate, and chalk, dolomite, and siderite belong to this group (Larsen et al., 1995). The focus here is on chalk. Chalk consists mainly of calcite (calcium carbonate) with minor amounts of biogenic fragments, clays and silica minerals that was deposited in a marine environment and went through diagenesis. It is classified as chalk or limestone depending on the hardness: Chalk is a soft material that can be manipulated with the fingers or scratched with a knife, whereas limestone is a harder material that can be manipulated with a knife or so hard that it cannot even be scratched by a knife.

Groundwater used as drinking water is abstracted in several countries from carbonate rock e.g. in UK about 55 % of the abstracted groundwater comes from carbonate rock aquifers (Besien et al., 2000), and in Denmark about 40 % of the drinking water is abstracted from carbonate rock aquifers (Sonnenborg, 2006). Figure 1A shows the distribution of chalk aquifer in Denmark and the waterworks wells that extract groundwater from these formations. When this figure is compared with figure 1B showing findings of pesticides in Danish waterworks wells it is clear that also the chalk aquifer is affected by pesticide contamination. It is therefore important to study the fate of these pesticides in aquifers and during transport to the aquifers, and thereby improve the predictions of duration of groundwater contamination and the effect of Natural Attenuation.

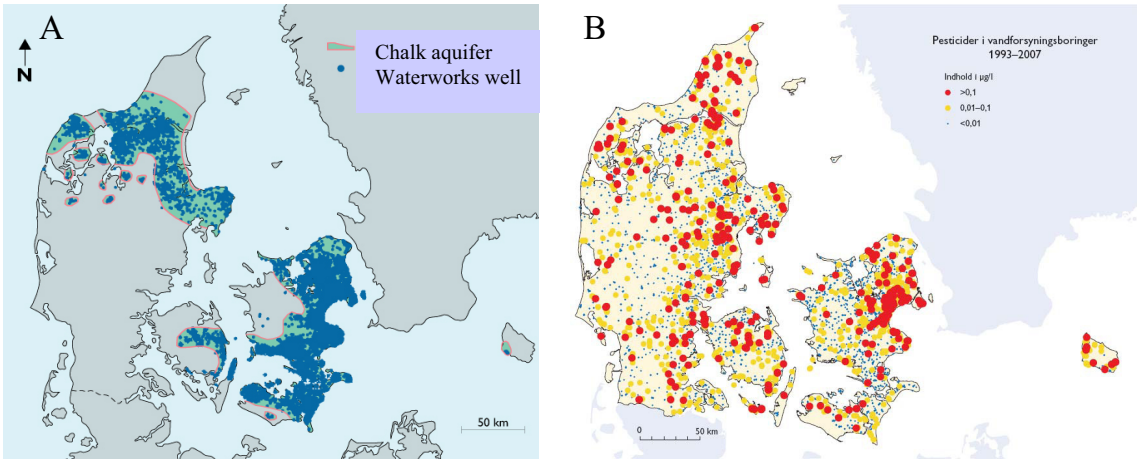


Figure 1: A) Distribution of chalk aquifers in Denmark and waterworks wells from where drinking water is extracted from the chalk aquifers (From Sonnenborg, 2006). B) Pesticides and their metabolites found in active waterworks wells during 1993-2007. Wells are included in map if pesticides or metabolites were found at least once in the well during that period (GEUS, 2009).

2.1 Transport of herbicides

Fractured chalk has dual porosity where the primary porosity is in the matrix and the secondary porosity in the fractures. The fractures may be enlarged by the water flow if the chalk is dissolved. Fractured chalk also has dual permeability. Primary permeability origins from the formation of the rock whereas secondary permeability is formed afterwards (Fetter, 2001). Fractures can increase the secondary permeability. The preferential flow can be both in matrix and fractures (figure 2). If the preferential flow is in the fractures, then transport of dissolved contaminants will be dominated by advection, whereas it will be dominated by diffusion if the preferential flow is in the chalk matrix. The porous chalk matrix is often fractured, and contaminants migrate from the fractures to the matrix primarily by diffusion (Wefer-Roehl et al., 2002). The duality increases the complexity in understanding the transport processes within the chalk.

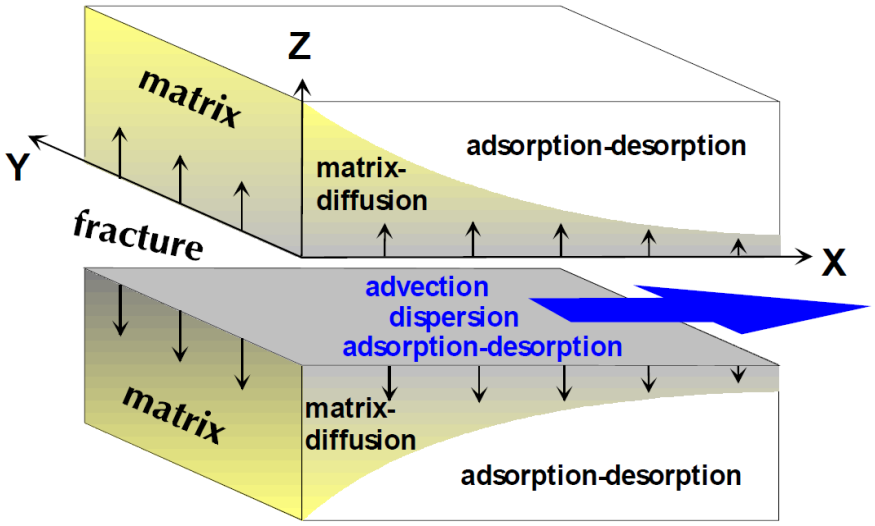


Figure 2: Transport processes in fractured media (From Fracflow, 2001)

Whether the preferential flow is in the fractures or in matrix has been discussed in literature for several years; at first fractures were thought to be the predominately flow path, but now the matrix flow is considered important (Mathias et al., 2005) since fracture flow can be rare, e.g. during a 6 year period it was only observed once in a 40 m thick unsaturated zone of Upper Chalk, UK (Wellings, 1984). Mathias et al. (2005) concluded that flow in matrix is significant at especially steady-state flow. The depth to the water table may also be a determining factor for the preferential flow since shallow groundwater sites

(~4 mbs) are likely to have preferential fracture flow whereas deeper (~18 mbs) are likely to have matrix flow (Haria et al., 2003).

Conclusively, preferential flow in unsaturated chalk defines the transport of herbicides to the aquifer. If the preferential flow is through fractures, the herbicides will be transported by advection; fast and with little retention. However, if matrix flow is preferential, then diffusion will dominate. Matrix flow may be very important in unsaturated chalk and limestone even though it is a fractured matrix and hence solutes transported with the water flow will diffuse into matrix and may sorb to matrix. Sorption can thereby increase the retention time and contribute to Natural Attenuation if the sorption is substantial.

3 Sorption of herbicides

Sorption can easily be investigated in batch equilibrium tests based on OECD Guideline 106 (OECD, 1993) where sorbate is added to a sorbent equilibrated with a liquid. This liquid can be e.g. groundwater, miliQ water, or $CaCl_2$. The choice of liquid may affect the sorption strength: $CaCl_2$ has no influence on sorption of uncharged pesticides, but strong influence on anionic pesticides (Clausen et al., 2001) such as mecoprop. The sorption of anionic pesticides to positive charged mineral surfaces decreases when using $CaCl_2$ whereas it increases when the mineral surfaces are negatively charged (controlled by pH) (Clausen et al., 2001). Sorption can be calculated as the distribution of sorbate between solid phase and solution at equilibrium:

$$K_d = \frac{C_s}{C_{eq}}$$

Where K_d is the linear distribution coefficient [L/Kg], C_s is the sorbate concentration associated with the sorbent [µg/Kg], and C_{eq} is the sorbate concentration in the solution at equilibrium [µg/L].

The retardation (R) of a compound due to sorption is defined as the compound travel velocity (v_s) divided by the water velocity (v_w), but can also be calculated based on the sorption (K_d), bulk density (ρ_b) and porosity (ε):

$$R = \frac{v_s}{v_w} = 1 + K_d \frac{\rho_b}{\varepsilon}$$

Sorption strength is often positively correlated to the organic carbon content (Arias-Estevez et al., 2008; Hiller et al., 2008; Payaperez et al., 1992). However, this is often very low (<0.1 %) in aquifer sediments and in chalk and limestone. The minerals – especially clays – and ironoxides then become important (Clausen et al., 2001; Clausen and Fabricius, 2001).

3.1 Sandy aquifer sediment

Sandy sediments consist mainly of quartz whereto isoproturon, atrazine, and mecoprop do not sorb at near-neutral pH (Clausen et al., 2001). Isoproturon and atrazine but not mecoprop also sorb to the clay mineral kaolinite (Clausen et al., 2001). Sorption of the herbicides to sandy aquifer sediments was studied by several (table 2). The aquifers were characterized by low total organic carbon (TOC) content ranging from 0.001 to 0.14 % and a varying sand content (table 2). Two studies (Janniche et al., **III**; Mouvet et al., 2004) included samples from both aerobic and anaerobic aquifers, and sorption was higher in the anaerobic samples probably because of changes in the organic carbon due to the reduced conditions which induced a more hydrophobic surface whereto nonionic compounds sorbs better (Clausen et al., 2004). The measured K_d values were in increasing order: mecoprop <0.01-0.26; atrazine <0.01-1.90; isoproturon <0.01-3.08; and acetochlor ~0.03-5.12 L/kg. The maximum K_d values of the four herbicides corresponds to retardation factors (R) of 2.6 (mecoprop), 12.4 (atrazine), 19.5 (isoproturon) and 31.7 (acetochlor) when assuming a sandy aquifer with a bulk density of 1.8 kg/l and a porosity of 0.3. Hence especially isoproturon and acetochlor may sorb strongly in sandy aquifer sediments.

Table 2: Sorption to sandy aquifer

Herbicide	K_d (l/kg)	TOC (%)	Depth (mbs)	N	C_0 (µg/L)	Method	Reference
Isoproturon	0.07-0.85	0.02-0.12	2.0-5.5	9	250	5 ml groundwater + 5 g solids	(Madsen et al., 2000)
	0.06-0.15	0.02	~5-6	2	25	Column experiment	(Tuxen et al., 2000)
	0.07-0.31	0.08-0.14	12.32-18.55	5	60-600	50 ml 0.01 M $CaCl_2$+ 15 g solid	(Rae et al., 1998)
	<0.01-1.69	0.07-0.11	19.20-19.45	4-5	1+50	5 ml groundwater + 5 g solid	(Janniche et al., **II**)
	0.05-3.08	0.001-0.055	1.7-47.1	12	50	5 ml groundwater + 5 g solid	(Mouvet et al., 2004)
Atrazine	N.D	0.08	6-7	1	300	10 ml 10^{-2} M $CaCl_2$ + 5 g soild	(Moreau and Mouvet, 1997)
	0.15-1.05	0.02-0.12	2.0-5.5	9	250	5 ml groundwater + 5 g solids	(Madsen et al., 2000)
	0.02-1.90	0.001-0.055	1.7-47.1	12	50	5 ml groundwater + 5 g solid	(Mouvet et al., 2004)
Mecoprop	0.07-0.26	0.02-0.12	2.0-5.5	9	250	5 ml groundwater + 5 g solids	(Madsen et al., 2000)
	0.296	0.044	3	1	100	5 ml 0.01 M $CaCl_2$+ 5 g solid.	(de Lipthay et al., 2007)
	0.00-0.04	0.02	~5-6	2	25	Column experiment	(Tuxen et al., 2000)
	N.S.-0.23	0.001-0.055	1.7-47.1	12	50	5 ml groundwater + 5 g solid	(Mouvet et al., 2004)
	<0.01-0.09	0.07-0.11	19.25-19.35	2	1+50	5 ml groundwater + 5 g solid	(Janniche et al., **II**)
Acetochlor	N.S.-5.12	0.001-0.12	1.7-47.1	14	50	5 ml groundwater + 5 g solid	(Janniche et al., **III**)

K_d: linear distribution coefficient, TOC: total organic content, N: number of samples. C_0: initial herbicide concentration, N.D.: not detectable.
N.S.: not significant as sorption was less than the standard deviation (approximately 0.03 L/kg).

3.2 Chalk and limestone

Sorption to limestone and chalk of the herbicides atrazine, mecoprop, isoproturon and acetochlor is in general low (Table 3) and is often related to impurities within the chalk and limestone settings such as clay and organic matter. Mecoprop sorbs though limited to calcite (0.20 L/kg), the major constituent of limestone and chalk, whereas atrazine and isoproturon does not (Clausen et al., 2001).

Sorption to chalk and limestone increased in the order mecoprop, atrazine, acetochlor, isoproturon with K_d values of b.d-0.17 L/kg for mecoprop, ≤0.09-0.35 L/Kg for atrazine, b.d.-0.49 L/kg for acetochlor and b.d.-0.55 L/kg for isoproturon (table 3). K_d values for acetochlor sorption to limestone and chalk are very scarce (Janniche et al., **III**). These maximum K_d values corresponds to R (retardation) values of 1.9 for mecoprop, 2.8 for atrazine, 3.5 for acetochlor and 3.8 for isoproturon (bulk density: 2 kg/L, porosity: 0.4). Hence, the herbicides may only be slightly retarded in the carbonate rock.

Quite large coefficient of variation (CV) has been reported in some of these sorption investigations (table 3), and as a consequence e.g. Roulier et al (2006) chose to use a CV of 50 % when modeling atrazine leaching in fractured limestone. These variations in sorption can not always be related directly to the factors normally expected to control sorption e.g. clay content, mineralogy and total organic carbon content. The nature of the organic carbon may be the controlling factor with more sorption to unoxidized chalk than to oxidized as Wefer-Roehl et al. (2001) observed large differences in sorption of aromatic compounds on chalk: sorption was 100- to 1000-fold higher in grey (unoxidized) chalk than in white (oxidized) chalk. Increased sorption in reduced environments has also been observed in soil and sediment samples (Clausen et al., 2004; Grathwohl, 1990; Janniche et al., **III**; Janniche et al., **II**). Wefer-Roehl et al. (2001) also found that sorption of organic compounds to fracture filling (mainly quartz, clays and calcite) was at the same low level as to oxidized chalk whereas it was imperceptible to the wall coatings (celestite, calcite and gypsum). Furthermore, sorption in chalk of a given organic contaminant may even be lower when in a mixture of compounds due to competitive sorption (Graber and Bonisover, 2003). This is often the case in both diffuse (e.g. agriculture) and point-source (e.g. landfills) contaminated aquifers that several pesticides and

metabolites are present at the same time. Hence, the total storage potential of carbonate rock is complicated to evaluate.

In summary, limestone and chalk has very limited sorption capacity for atrazine, mecoprop, isoproturon and acetochlor, compared to other geological settings with high clay or organic carbon content. The herbicides will therefore remain bioavailable, and with only limited retardation be subject to leaching. In the sandy aquifer retardation of especially acetochlor but also isoproturon can be substantial, especially under anaerobic conditions whereas mecoprop sorption remains almost as low as in limestone and chalk.

Table 3: Sorption to chalk and limestone

Herbicide	K_d (l/kg)	TOC (%)	Depth (mbs)	N	C_0 (µg/L)	Method	Reference
Isoproturon	≤0.02	<0.1	1.71-7.85	3	1000	20 mL 2 mM $CaCl_2$ + 20 g <2 mm solid	(Besien et al., 2000)
	0.02-0.46	<0.1-0.38	2.1-9.9	4	1000	3 mL 10 mM $CaCl_2$ + 3 g <3 mm solid	(Johnson et al., 1998)
	0.16	0.003	42.3-45.5	1	250	5 mL GW +2 g <2 mm solid	(Madsen et al., 2000)
	0.05-0.12 (outlier of 0.55)	0.7-2.9 (outlier: 0.1)	3-12	11	381	10 mL 0.01 M $CaCl_2$ + 5 g <2 mm solid	(Coquet et al., 2004)
	b.d.-0.25	0.6-5.4	4.5-26.4	10	1+50	5 mL GW + 5 g <2 mm solid	(Janniche et al., **II**)
	≤0.28	-	1.00-15.56	8	50	5 mL GW + 5 g <2 mm solid	(Mouvet et al., 2004)
Atrazine	≤0.09	0.02	2.8-3.2	1	2500	7 mL miliQ water chalk saturated + 10 g solid	(Kristensen et al., 2001a)
	0.15	0.003	42.3-45.5	1	250	5 mL GW + 2 g <2 mm solid	(Madsen et al., 2000)
	0.09-0.15 (outlier of 0.35)	0.7-2.9 (outlier: 0.1)	3-12	11	3590	10 mL 0.01 M $CaCl_2$	(Coquet et al., 2004)
	0.09		1-3				
	0.05	-	3-6	4	50	5 mL spring water + 5 g solid	(Roulier et al., 2006)
	0.03		6-8				
	Negligible		>8				
	≤0.27	-	1.00-15.56	8	50	5 mL GW +5 g <2 mm solid	(Mouvet et al., 2004)
Mecoprop	b.d.	0.02	2.8-3.2	1	2500	7 mL miliQ water chalk saturated + 10 g solid	(Kristensen et al., 2001a)
	0.17	0.003	42.3-45.5	1	250	5 mL GW +2 g <2 mm solid	(Madsen et al., 2000)
	b.d.-0.15	0.6-5.4	4.5-26.4	10	1+50	5 mL GW +5 g <2 mm solid	(Janniche et al., **II**)
	≤0.13	-	1.00-15.56	8	50	5 mL GW +5 g <2 mm solid	(Mouvet et al., 2004)
Acetochlor	<0.01-0.49	0.6-5.4	1.0-26.4	14	1+50	5 mL GW +5 g <2 mm solid	(Janniche et al., **III**)

K_d: linear distribution coefficient. TOC: total organic content. N: number of samples. C_0: initial herbicide concentration. GW: groundwater. N.D.: not detectable. N.S.: not significant as sorption was less than the standard deviation (approximately 0.03 L/kg).

4 Degradation

Microorganisms are capable of degrading many herbicides by enzyme catalyzing processes resulting in reduced complexity of the chemical. The degradation is called mineralization if the herbicide is degraded to CO_2 and inorganic products. Degradation can either induce growth (multiplication) as the compound is used as C- and energy source or non-proliferation (non-growth) as the microorganisms degrade without gaining energy or nourishment (co-metabolism). Mineralization is often growth-linked so the number of degrading bacteria increases as the compound concentration decreases (Alexander, 1999). However, there is no general rule towards how much of the compound is incorporated in the bacteria and how much is excreted as CO_2 as it can vary a lot depending on e.g. the compound and the microorganisms present (Alexander, 1999).

4.1 Importance of initial concentration

Essential prerequisites for the biodegradation are the presence of bacteria with enzymatic systems coding for degradation of the specific herbicide and bioavailable herbicide e.g. dissolved in water. Realistic herbicide concentrations should be used when simulating natural degradation of herbicides whether it is in topsoil or in deep subsurface, and this has been known for at least 30 years (Boethling and Alexander, 1979). Effect of initial concentration on the degradation kinetic has been documented both in samples from vadose zone and in aquifer sand (de Lipthay et al., 2007; Helweg et al., 1998; Janniche et al., **I**; Reffstrup et al., 1998; Torang et al., 2003). The degradation in soil is often growth-linked at pesticide concentrations above 1 mg/kg and non-growth-linked below this concentration (Formsgaard, 2004). In the subsurface it might be more complex.

Janniche et al. (**I**) studied the effect of six different initial concentrations (0.5-100 µg/kg) in unsaturated *limestone* on the mineralization of acetochlor, isoproturon, and mecoprop (figure 3). Acetochlor mineralization was relatively low (<2.5 %) at all concentrations resulting in half-lives ($T_{½}$) of 19-142 years. Isoproturon mineralization was highest at concentrations of 0.5, 1 and 20 µg/kg but still with notable long $T_{½}$ of approximately 6 years, and not mineralized at 100 µg/kg. Mecoprop was substantially mineralized (≥20 %) at all concentrations with sigmoid mineralization curves that reflected first order mineralization and half-lives ($T_{½}$) of 0.5-2.0 years. Conclusively, in limestone there was no clear effect

of initial concentrations of 0.5-100 µg/kg, but there might be at higher concentrations.

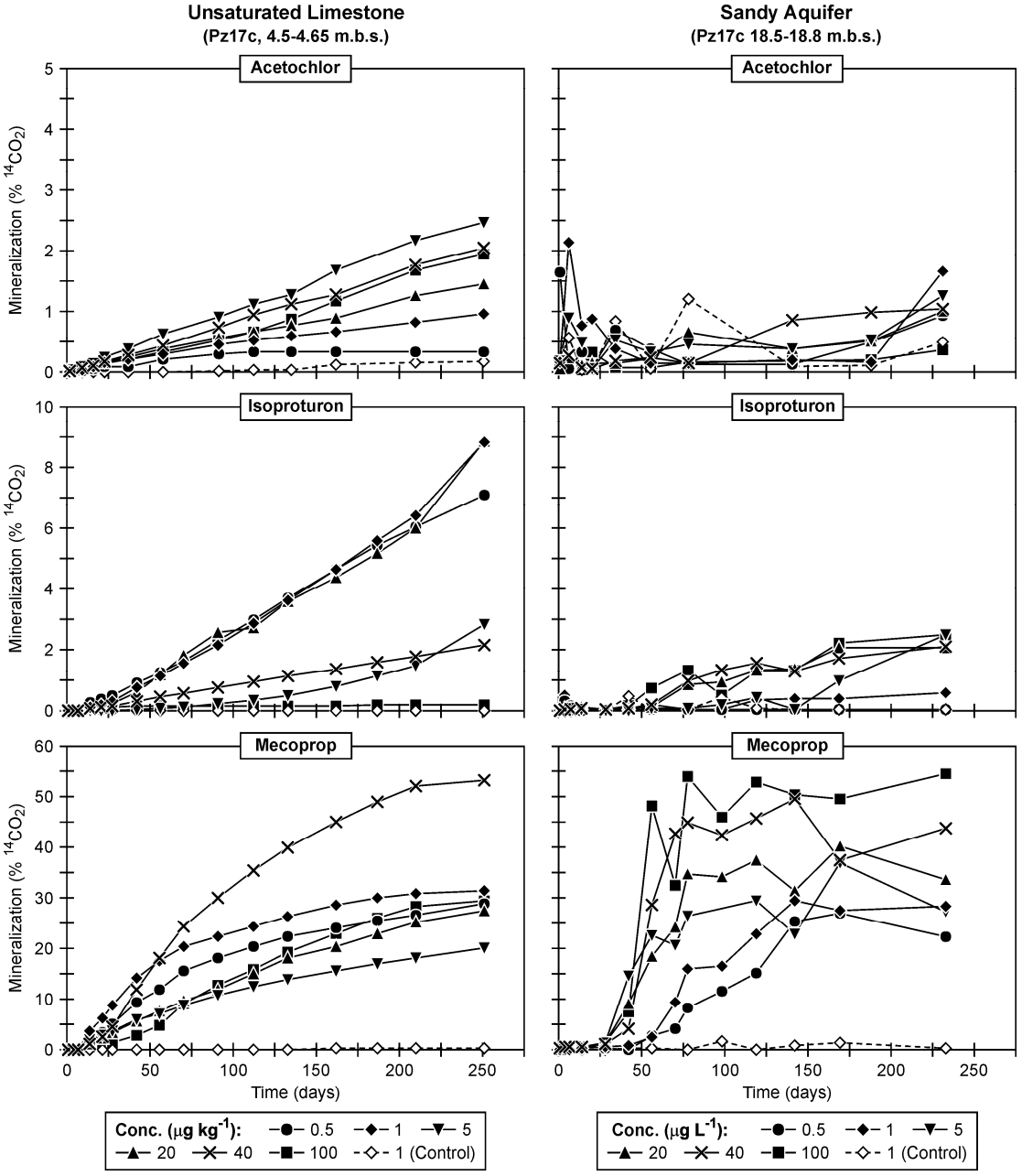


Figure 3: Mineralization kinetics at six different initial concentrations in unsaturated limestone (left) and in sandy aquifer (right). Note the different scales on y-axis. (Janniche et al., I)

In *sandy aquifers*, effect of initial concentration was observed (figure 3) for mecoprop, whereas no conclusion could be drawn for acetochlor and isoproturon because these were not substantially mineralized (Janniche et al., **I**). The amount mecoprop mineralized in 231 days increased with increasing initial

concentrations, and incubations with 0.5 and 1 µg/L had twice as long lag phases than incubations with 5-100 µg/L. Mecoprop degradation was also significantly faster at high (25-100 µg/L) initial concentrations than at low (1-10 µg/L) initial concentrations in a herbicide pre-exposed aquifer (Torang et al., 2003). In accordance, de Lipthay et al. (2007) found mecoprop half-times of 0.7-1.9 years at extremely high (50-10 000 µg/L) concentrations and significant longer half-times of >4 years at low (0.10-10 µg/L) concentrations. Furthermore, in a sandy sediment from a Danish shallow aquifer Torang et al. (2003) investigated degradation kinetic at very low concentrations (0.2-100 µg/L corresponding to about 0.25-125 µg/kg) of the two phenoxy acids mecoprop and 2,4-D and found different threshold concentrations for each herbicide above which the degradation was growth-linked, and non-growth-linked below. This indicates that even herbicides belonging to the same group may be degraded by different kinetics. Figure 4 shows the degradation over time of 2,4-D at five different initial concentrations (Torang et al., 2003) and it is obvious that the incubations with 0.2 and 1 µg/L follows non-growth degradation kinetic whereas the incubations at the higher concentrations are growth-linked.

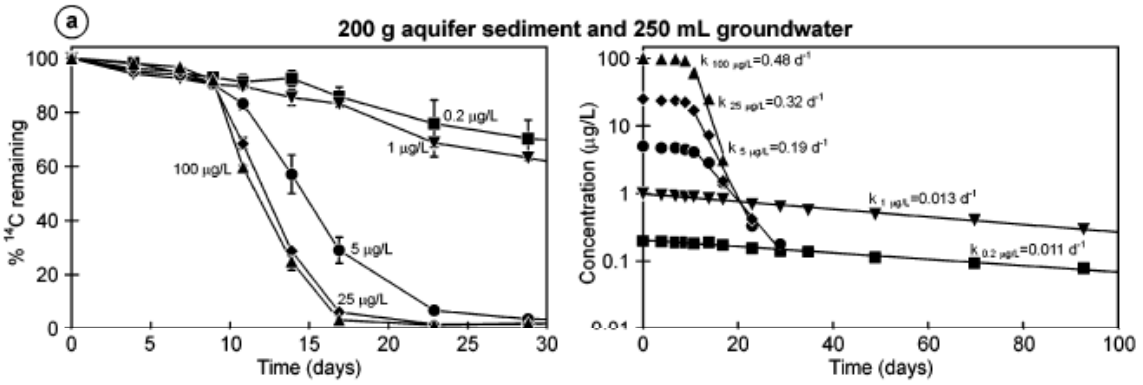


Figure 4: Degradation of 2,4-D at different initial concentrations given as % ^{14}C remaining (left) and concentration (rigth). 1st order kinetics were used to calculate degradation rates. (From Torang et al., 2003)

In brief, degradation in sandy aquifers may be fast at high concentrations, but slow at low concentrations and therefore degradation rates can be overestimated if unrealistic high concentrations are used. E.g. based on the 1st order degradation rates (k) from figure 4 a half life ($T_{½}$= ln2/k) of 1.4 days can be calculated, hence 2,4-D is readily degraded in shallow aerobic aquifers. However if the lowest rate ($k_{0.2\ µg/L}$) is used instead of the highest ($k_{100\ µg/L}$) then the $T_{½}$ is 63 days, and 2,4-D is only poorly degradable.

4.2 Soil and shallow vadose sediments

The herbicides mecoprop, isoproturon, atrazine and acetochlor are all degradable in topsoil and shallow sub-surface soil under aerobic conditions (Alletto et al., 2006; Baran et al., 2004; Buss et al., 2006; Charnay et al., 2005; Dictor et al., 2008; Issa and Wood, 2005; Johnson et al., 2000; Mouvet et al., 2004; Taylor et al., 2005). However, atrazine is not always easily mineralized in topsoil as Larsen et al. found limited mineralization (3 %) in surface soil. In some cases atrazine and isoproturon were not mineralized below 0.8 mbs (Larsen et al., 2000; Wood et al., 2002). Acetochlor, atrazine and isoproturon are also degradable under anaerobic conditions (Issa and Wood, 2005; Larsen et al., 2000; Loor-Vela et al., 2003) whereas mecoprop is not (Buss et al., 2006).

That the herbicides can be degraded and even mineralized in shallow sediments does not necessary imply that they can also be degraded in deep subsurface or in other geological settings. However, it documents that microorganisms which can degrade herbicides are naturally present.

4.3 Groundwater

Two kinds of microorganisms are present in aquifers: planktonic (free-living) and benthic (attached to solid aquifer materiel), but most microorganisms can shift between the two (Goldscheider et al., 2006). Benthic microorganisms dominate in oligotrophic aquifers in terms of biomass and activity, since the majority of the planktonic cells are inactive subsets of benthic organisms (Goldscheider et al., 2006; Griebler and Lueders, 2009). Hence, mineralization of herbicides is most likely done by benthic bacteria. Johnson et al. (1998), however, found degradation of isoproturon only in groundwater with sterile chalk and not in chalk with sterile groundwater, and Besien et al. (2000) found groundwater from a Chalk aquifer had free-living bacteria capable of degrading isoproturon. This indicates that the aquifer environment is complex and the metabolism of planktonic bacteria needs to be studied further. However, since bacteria are mainly associated with fine particles (Holm et al., 1992), addition of biomass support material to groundwater incubations often increases the degradation compared to incubations with groundwater alone (e.g. Albrechtsen et al., 1996; Albrechtsen et al., 1997; Holm et al., 1992; Johnson et al., 1998; Poeton et al., 1999), apparently because the groundwater bacteria require a surface for attachment before multiplying and/or producing the right enzymes for

herbicide degradation (Chilton et al., 2005). Besides, mineral heterogeneity in pristine aquifers is believed to affect microbial community structure and diversity since different minerals selected for different subdivisions of bacteria (Boyd et al., 2007).

Though herbicide degradation in groundwater (or in groundwater with sterile biomass support material) is less studied than in aquifer sediment there might appear to be some trends which can be concluded from laboratory studies.

4.3.1 Atrazine

Groundwater from a shallow aerobic sandy aquifer could not degrade atrazine (100 µg/L) during 539 days at 10 ˚C (Klint et al., 1993), whereas groundwater from sandstone, limestone and chalk aquifers incubated with sterile material from the aquifer could degrade but not mineralize atrazine (100 µg/L) with half-lifes of 50-100 years (Johnson et al., 2000; Johnson et al., 2003). Only one out of 18 incubations with groundwater sampled from 7 piezometers in deep (14-42 mbs) aerobic sandy aquifers could mineralize 1 µg/L atrazine (figure 5) (Janniche et al. **IV**). This incubation was with biomass support material of rinsed, sterile, well defined quartz sand. Two years later the piezometer with atrazine mineralization no longer harbored groundwater with atrazine mineralization potential (Janniche et al. **IV**) nor did 6 additional piezometers from the same catchment (data not shown). *Hence, atrazine degradation appears to be rare.*

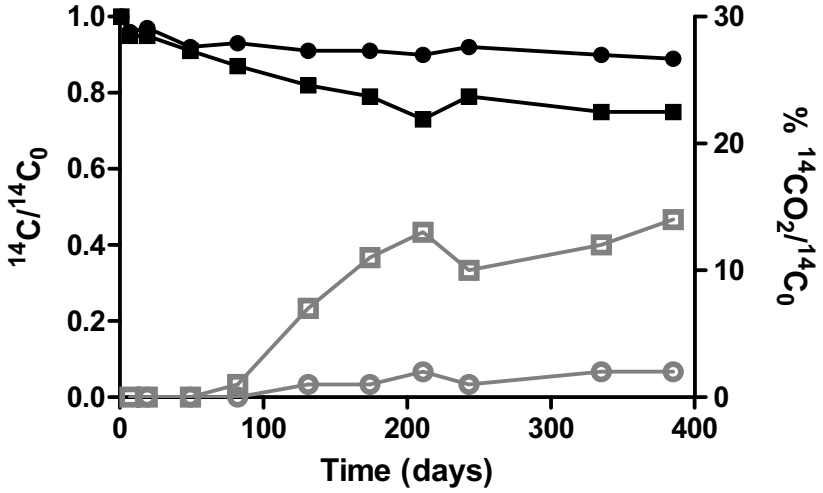


Figure 5: Atrazine degradation ($^{14}C/^{14}C_0$; closed symbols) and mineralization (% $^{14}CO_2/^{14}C_0$; open symbols) (Modified from Janniche et al., IV).

4.3.2 Acetochlor

Janniche et al. **IV** is apparently the only study on acetochlor degradation in groundwater and *no mineralization was observed in any of the 13 groundwater samples collected from a sandy aquifer* (data not shown).

4.3.3 Isoproturon

In an investigation of both degradation and mineralization of isoproturon in chalk from unsaturated and saturated zone (but incubated identically and with groundwater) mineralization was not observed in any samples (<1 % after 4 moths at 20 ˚C), but degradation was observed (to below detection limit of 10 µg/L within 14 days) when the chalk was incubated with non-sterile filtered groundwater instead of sterile-filtered groundwater (Johnson et al., 1998). Hence, the bacteria inherent in the chalk were not able to degrade isoproturon whereas the bacteria in the groundwater were. However, the groundwater bacteria needed a biomass support material in order to initiate the degradation (Johnson et al., 1998). Groundwater sampled from sandstone, limestone and chalk aquifers incubated with sterile material from the aquifer was able to degrade isoproturon (100-200 days incubation at 20 ˚C at 100 µg/L) with half-lives of 34-240 days in sandstone, 204-320 days in chalk and 247-759 days in limestone (Johnson et al., 2000; Johnson et al., 2003). Isoproturon at 1 µg/L was also mineralized in groundwater from one out of 13 investigated piezometers with up to 53% in 400 days (Janniche et al. **IV**; data not shown). *In conclusion, isoproturon is rarely mineralized but is degradable by free-living bacteria yet with spatial variability.*

4.3.4 Mecoprop

Mecoprop was degraded in a groundwater suspension (aerobic aquifer sediment and groundwater sampled 5-7 mbs (N=16) mixed in a ration of 2.0 l groundwater to 1.0 kg sediment, and after 1 minute of sedimentation the water phase was transferred and used in the degradation study) with initial concentrations of 65-1400 µg/L (10 ˚C for 200 days), but it is unclear whether the degradation potential should be ascribed benthic or planktonic bacteria – or both (Heron and Christensen, 1992). Nevertheless, mecoprop can be degraded in groundwater without aquifer sediment; mecoprop at 100 µg/L was degraded (approximately 100 %) in 80 days in groundwater from a shallow sandy aquifer, groundwater, however, sampled one year later from the same location could not degrade mecoprop (Klint et al., 1993). Only groundwater sampled from sandstone, and not limestone and chalk aquifers incubated with sterile material from the aquifer

was able to degrade mecoprop (up to about 80 %) but not mineralize (approximately 200 days incubation at 20 ˚C at 100 µg/L) (Johnson et al., 2000; Johnson et al., 2003). *Hence, mecoprop is degradable in some groundwater at high concentrations.*

Since degradation and even mineralization of atrazine, isoproturon, and mecoprop can occur in groundwater it should be even more pronounced in sandy aquifer sediment with more bacteria. However, the herbicide degradation potential may not be widespread as it is not observed in all groundwater samples.

4.4 Chalk and limestone

Most of the degradation studies in chalk and limestone were performed on samples from vadose zone occasionally added sterile groundwater from the aquifer beneath (table 4). Few studies included the deep subsurface (>10 mbs), and only two studies included samples from >21 mbs (Janniche et al., **I**; Johnson et al., 1998). Few studies used low (<10 µg/kg) initial concentrations, therefore studies have been included in this summary regardless of the initial concentration as well as studies on shallow subsurface.

4.4.1 Atrazine

Atrazine is very recalcitrant in chalk and limestone and was not degraded in samples from neither shallow nor deep subsurface when incubated at 10-400 µg/kg (table 4), – not even in samples taken beneath an atrazine degrading soil (0-0.5 mbs) (Mouvet et al., 2004). However, when incubated at 5 mg/kg 35-45 % was mineralized in 6 months in samples from both shallow and deep subsurface. This is not a concentration reflecting diffuse contamination, but rather a point source. Hence, atrazine may not be degradable at concentrations in the µg-range.

4.4.2 Acetochlor

Acetochlor degradation in subsurface has rarely been studied previously (Dictor et al., 2008; Mills et al., 2001; Taylor et al., 2005), and only Janniche et al. (**III**) have studied the degradation in limestone. 34 limestone samples were incubated at 10 µg/kg or at a more realistic concentration of 1 µg/kg (table 4) to investigate mineralization which was clearly faster in samples incubated at the high initial concentration (samples named C1 and C2 in figure 6) than in samples incubated with the low concentration (samples named Pz14 and Pz17c in figure 6).

Acetochlor mineralization was evident, though slow, in only 12 samples, with half-lifes from 3.9 to 27 years.

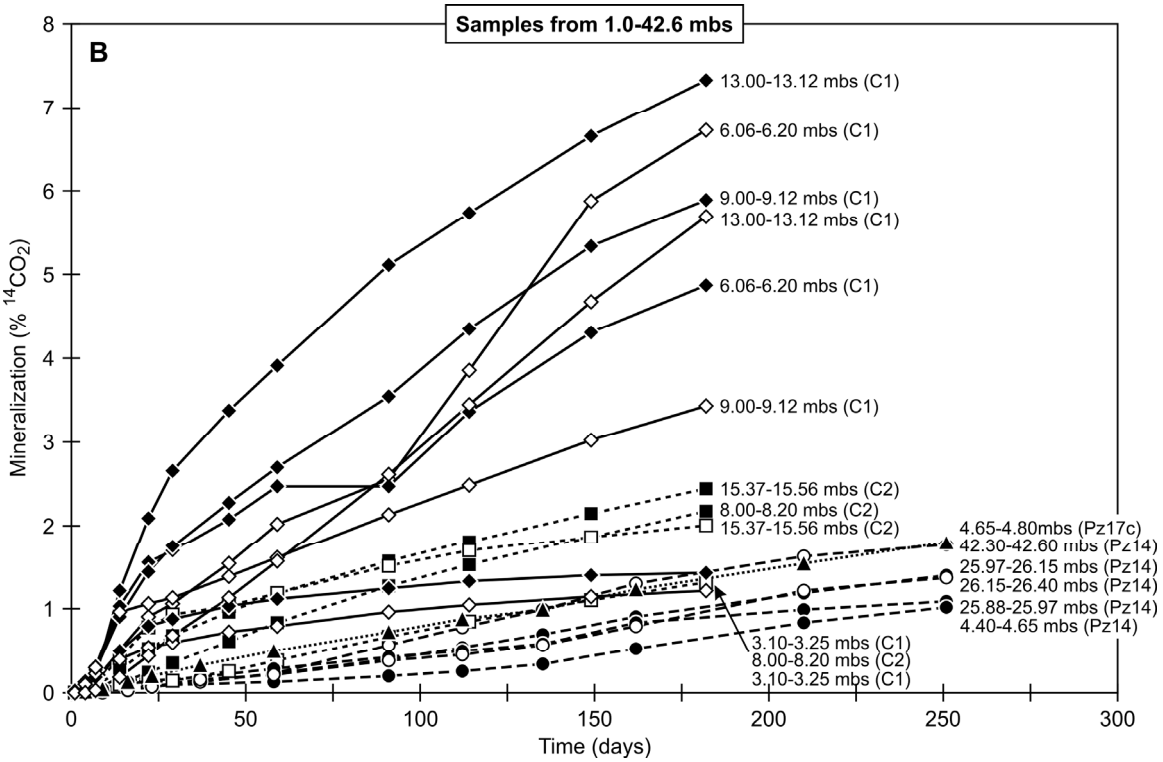


Figure 6: Mineralization of acetochlor (% $^{14}CO_2$) in unsaturated limestone samples (included if mineralization was above level in autoclaved control incubations). Open symbols represents duplicates (Modified from Janniche et al., III).

4.4.3 Isoproturon

Compared to the three other herbicides, more studies have been done on isoproturon degradation in carbonate rock, though rarely at low (<10 µg/kg) concentrations (table 4). Only two studies used low initial concentrations: Kristensen et al. (2001b) used 6 µg/kg and Janniche et al. (**I**) used 1 µg/kg. Furthermore, only two studies included limestone or chalk samples from below 20 mbs (Janniche et al., **I**; Johnson et al., 1998).

Isoproturon was degradable in most studies from unsaturated zone where up to approximately 78 % was degraded during some 260 days incubation at 20 °C and 200-300 µg/kg (Johnson et al., 2003), but not in all (table 4). Surprisingly these samples with no degradation were primarily from shallow depths (<10 mbs) which supports the finding of Janniche et al. (**I**) that mineralization in limestone not simply decreased with increasing depths: In samples from 1.5-42.6 mbs substantial subsurface mineralization was evident; 21 % isoproturon was

mineralized in 6.7-7.0 mbs though there was no significant mineralization (0.1 %) in the shallowest sample (Janniche et al., **I**).

There was substantial isoproturon degradation in samples from unsaturated limestone incubated with sterile groundwater (Johnson et al., 2003), hence isoproturon degrading bacteria were present in the unsaturated limestone that could degrade under saturated conditions. However, in another study where unsaturated chalk collected at 4.42-4. 88 mbs was incubated without groundwater there was no evidence of isoproturon degradation, yet when unsaturated chalk collected at 10.7 mbs from that same field site was incubated with sterile groundwater degradation was evident with a half-life of 150 days (Johnson et al., 2000). So either the isoproturon degrading bacteria are not evenly distributed in the unsaturated chalk or they are more active in the deeper layers or degradation is stimulated by the presence of sterile groundwater. The later might be the dominating factor since a water phase would increase the mobility of the bacteria and the availability of isoproturon.

Only five samples from saturated chalk or limestone was investigated; and isoproturon degrading bacteria where present in sufficiently high numbers to initiate degradation in all samples but one. Complete degradation was only observed in the two samples from the shallow (2.6-4.45 mbs) chalk aquifer (max 4.2 ± 0.3 %) incubated with 6 µg/L (Kristensen et al., 2001b); degradation but not mineralization was observed in the two deep subsurface samples incubated with 100-200 µg/L (table 4).

4.4.4 Mecoprop

Though Johnson et al. (2000; 2003) did not observe mecoprop degradation in limestone and chalk from neither the unsaturated nor saturated zone at 300-400 µg/kg it was mineralized at 1-10 µg/kg in at least one sample from each mineralization study (table 4). Mineralization may be faster in unsaturated shallow (<10 mbs) samples than in deep subsurface samples, but the mineralization did not simply decrease with increasing depths in 26 limestone samples from 1.5-42.6 mbs (Janniche et al., **I**). 0.7-1.0 % was mineralized in the uppermost sample and in the deepest sample respectively, but substantial mineralization was evident in samples in-between e.g. 4.50-4.65 mbs had 32 % mineralization (Figure 7). Mecoprop was also mineralized in two samples from shallow aquifers; up to 33 % was mineralized during 258 days incubation at 10˚C

(Kristensen et al., 2001b). High coefficient of variance (CV) was encountered in the two aquifer chalk samples (57 % and 73 %) and spatial heterogeneity of the microbial activity was suggested as an explanation. Half-lifes between 1.1- 43.4 years were reported for the samples with mineralization, however, mineralization was not observed in every sample.

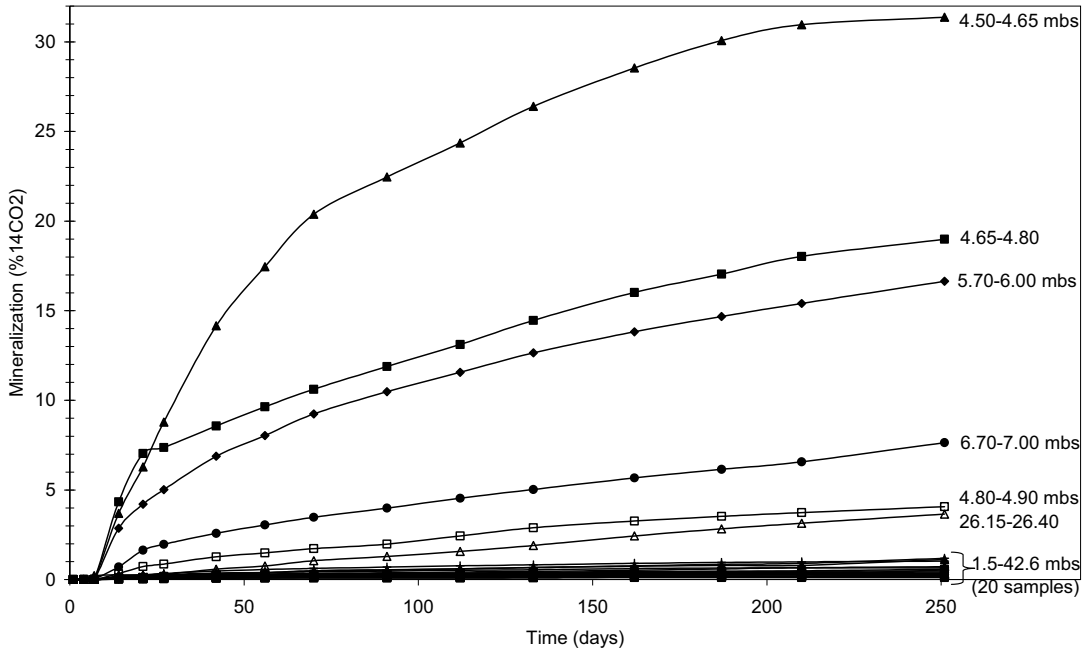


Figure 7: Mineralization of mecoprop in 26 limestone samples (1.5-42.6 mbs) incubated with 1µg/kg (autoclaved control incubations had 0.4 %) (based on Janniche et al., I).

4.4.5 Summary on degradability in chalk/limestone

The four herbicides can all be degraded and even mineralized in chalk and limestone, and even in samples from deep subsurface (>10 mbs). However, they are not always degraded, and mineralization did not just simply decrease with increasing depths, but indeed sub-surface mineralization was evident, though slow. Fissures in limestone may be important for downward transport of both nutrients and degrading microorganisms and may have caused these active subsurface zones (Janniche et al., **I**). Furthermore, the majority of microbial activity is associated with fractures in chalk because the typical pore-throat diameters of the chalk exclude most metabolic active microorganisms (Johnson et al., 1998; Kristensen et al., 2001b).

Table 4: Degradation of herbicides in subsurface chalk or limestone.

Herbicide	Unsat or Sat.	Depth (mbs)	N	C_0 (ug/L)	C_0 (ug/Kg)	Time (days)	Temp (˚C)	Mineralization ± SD (%)	Degradation (%)	T½ (year)	Ref
Atrazine	Unsat.	1.0-9.1	6	-	10	182	15	No	n.a.	None	(Mouvet et al., 2004)
	Unsat	3.4-8.3	8	-	5000	~180	25	n.a.	~35-40	n.a.	(Issa and Wood, 1999)
	Unsat.[a]	3.6 + 5.2	2	100	300-400	~260	20	n.a.	No	None	(Johnson et al., 2003)
	Unsat.	4.6-10.0	7	-	100	28	28	N.S.	n.a.	None	(Charnay et al., 2005)
	Unsat.[a]	10.3-10.75	1	100	375	~210	20	n.a.	No	None	(Johnson et al., 2000)
	Unsat	12.4-16.3	7	-	5000	~180	25	n.a.	~35-45	n.a.	(Issa and Wood, 1999)
	Unsat.	13.0-15.6	2	-	10	182	15	No	n.a.	None	(Mouvet et al., 2004)
	Sat.[a]	18.8-19.3	1	100	375	~210	20	n.a.	No	None	(Johnson et al., 2000)
Mecoprop	Unsat.	1.0-9.1	6	-	10	182	15	1.8-5.7	n.a.	1.3/10.8-4.8/27.1[b]	(Mouvet et al., 2004)
	Unsat.	1.40-1.55	1	5.5	6	258	10	5.0±0.5	n.a.	n.a.	(Kristensen et al., 2001b)
	Unsat.	1.5-9.0	17	7	1	251	10	N.S-31.4	n.a.	1.1-none	(Janniche et al., **I**)
	Sat.[a]	2.60-2.80	1	5.5	6	258	10	9.7±5.5	n.a.	n.a.	(Kristensen et al., 2001b)
	Unsat.[a]	3.6 + 5.2	2	100	300-40(	~260	20	n.a.	No	None	(Johnson et al., 2003)
	Sat.[a]	4.30-4.45	1	5.5	6	258	10	17.3±12.7	n.a.	n.a.	(Kristensen et al., 2001b)
	Unsat.[a]	10.3-10.75	1	100	375	150	20	n.a.	No	none	(Johnson et al., 2000)
	Unsat.	13.0-15.6	2	-	10	182	15	1.7-2.3	n.a.	38.1-43.4[c]	(Mouvet et al., 2004)
	Sat.[a]	18.8-19.3	1	100	375	150	20	n.a.	No	None	(Johnson et al., 2000)
	Unsat.	21.8-42.6	9	7	1	251	10	N.S.-5.6	n.a.	9.5-none	(Janniche et al., **I**)
Acetochlor	Unsat.	1.0-9.1	6	~100	10	182	15	N.S.-6.7	n.a.	5.6-none	(Janniche et al., **III**)
	Unsat.	1.5-9.0	17	7	1	251	10	N.S.-1.8	n.a.	27-none	(Janniche et al., **III**)
	Unsat.	13.0-15.6	2	~100	10	182	15	2.0-7.3	n.a.	3.9-7.8/21.0	(Janniche et al., **III**)
	Unsat.	21.8-42.6	9	7	1	251	10	N.S.-2.0	n.a.	27-none	(Janniche et al., **III**)

Table 4 continued

Herbicide	Unsat or Sat.	Depth (mbs)	N	C_0 (ug/L)	C_0 (ug/Kg)	Time (days)	Temp (˚C)	Mineralization ± SD (%)	Degradation (%)	T½ (year)	Reference
Isoproturon	Unsat.	1.0-9.1	6	-	10	182	15	N.S-10.2	n.a.	3.3-none	Mouvet et al 2004
	Unsat.	1.40-1.55	1	5.5	6	258	10	2.1±0.1	n.a.	n.a.	(Kristensen et al., 2001b)
	Unsat.	2.1	1	100 (d) 200 (m)	300 (d) 100 (m)	121	20	No (< 1)	Yes	0.07-0.12	(Johnson et al., 1998)
	Sat.[a]	2.60-2.80	1	5.5	6	258	10	4.2±0.3	n.a.	11.4[f]	(Kristensen et al., 2001b)
	Unsat.	3.1	1	100	300 (d) 50 (m)	121	20	No (1)	No	None	(Johnson et al., 1998)
	Unsat	3.4-8.3	8	-	5000	~180	25	n.a.	~40-60	n.a.	(Issa and Wood, 1999)
	Unsat.	1.5-9.0	17	8	1	251	10	N.S.-21.4	n.a.	2.1-none	(Janniche et al., I)
	Unsat.[a]	3.6	1	100	300-400	~260	20	n.a.	~44	n.a.	(Johnson et al., 2003)
	Sat.[a]	4.30-4.45	1	5.5	6	258	10	4.1±1.4	n.a.	11.7[f]	(Kristensen et al., 2001b)
	Unsat	4.42-4.88	1	100	~30	200	20	n.a.	No	None	(Johnson et al., 2000)
	Unsat.[a]	5.2	1	100	300-400	~260	20	n.a.	~78	n.a.	(Johnson et al., 2003)
	Sat.[a]	7.2	1	100	300 (d) 50 (m)	121	20	No (< 1)	No	None	(Johnson et al., 1998)
	Unsat.	9.9	1	100 (d) 200 (m)	300 (d) 100 (m)	125	20	No (< 1)	Yes	0.42-none (d)	(Johnson et al., 1998)
	Unsat.[a]	10.3-10.75	1	100	375	~210	20	n.a.	Yes	0.41 (d)	(Johnson et al., 2000)
	Unsat.[e]	12.1-12.9	1	-	~300-350	162	16	n.a.	~18 %	n.a.	(Besien et al., 2000)
	Unsat.	13.0-15.6	2	-	10	182	15	7.3-9.0	n.a.	2.2/5.5[d]-4.1	Mouvet et al 2004
	Sat.[a]	18.8-19.3	1	100	375	~210	20	n.a.	Yes	0.63 (d)	(Johnson et al., 2000)
	Unsat.	20.7	1	100 (d) 200 (m)	300 (d) 100 (m)	125	20	No (< 1)	Yes	0.17-none (d)	(Johnson et al., 1998)
	Unsat.	21.8-42.6	9	8	1	251	10	N.S.-0.4	n.a.	95-none	(Janniche et al., I)
	Sat.[a]	28.0	1	100 (d) 200 (m)	300 (d) 100 (m)	125	20	No (< 1)	Yes	0.16-3.6 (d)	(Johnson et al., 1998)

Unsat. or sat.: sample from unsaturated or saturated zone. N: number of environmental samples. C_0: initial concentration. $T_{½}$: half-life. [a]: sterile groundwater or MiliQ-water added to incubation. [b]: $T_{½}$ calculated for t<22 days/t>22 days. [c]: calculated for t>14 days (correlation to 1st order mineralization not possible at t<14 days). [d]: calculated for t<45 days/t>45 days. [f]: calculated as $(\ln(2)\times Time)/\ln(C/C_0)$. (d): degradation investigation, (m): mineralization investigation. N.S.: not significant.

4.5 Sandy aquifer

Degradation studies in deep (>10 mbs) sandy aquifers with herbicide concentrations of ≤1 µg/L are scarce. In general most degradation studies with sandy aquifer sediment are performed at high initial concentrations (≥25 µg/L) (table 5).

4.5.1 Atrazine

Atrazine is a very recalcitrant compound that is not degradable in sandy aquifers regardless of the redox conditions and initial concentration (table 5). Only McMahon (1992) found that the ethyl (C_2H_5) group on atrazine could be degraded in sandy aquifer with a half life of 3.5 to more than 42 years, this study, however, was performed with a very high concentration of 4.5 mg/l. Neither field injection studies showed evidence of atrazine degradation (Papiernik and Spalding, 1998; Rugge et al., 1999; Widmer and Spalding, 1995).

4.5.2 Acetochlor

Acetochlor was not degraded in four European sandy aquifers (both aerobic shallow and deep samples and shallow anaerobic samples) despite an almost two-year long incubation period when incubated with 50 µg/L, however when incubated at 1 µg/L acetochlor mineralization (3.0-5.6 %) was evident in five deep subsurface samples (19.25-19.53 mbs) from one of the four aquifers (table 5). Furthermore, the acetochlor mineralization was only slightly decreased when incubated anaerobically (figure 8). The three samples were incubated under both aerobic and anaerobic conditions because these samples were grayish indicating reduced conditions and later test confirmed microaerobic conditions. The results indicated that acetochlor can be mineralized under anaerobic or microaerobic conditions (Janniche et al., **III**). This transition zone between aerobic and anaerobic sediment might have induced an ecotone with increased microbial metabolic activity as also seen in the fringe of groundwater pollution plumes.

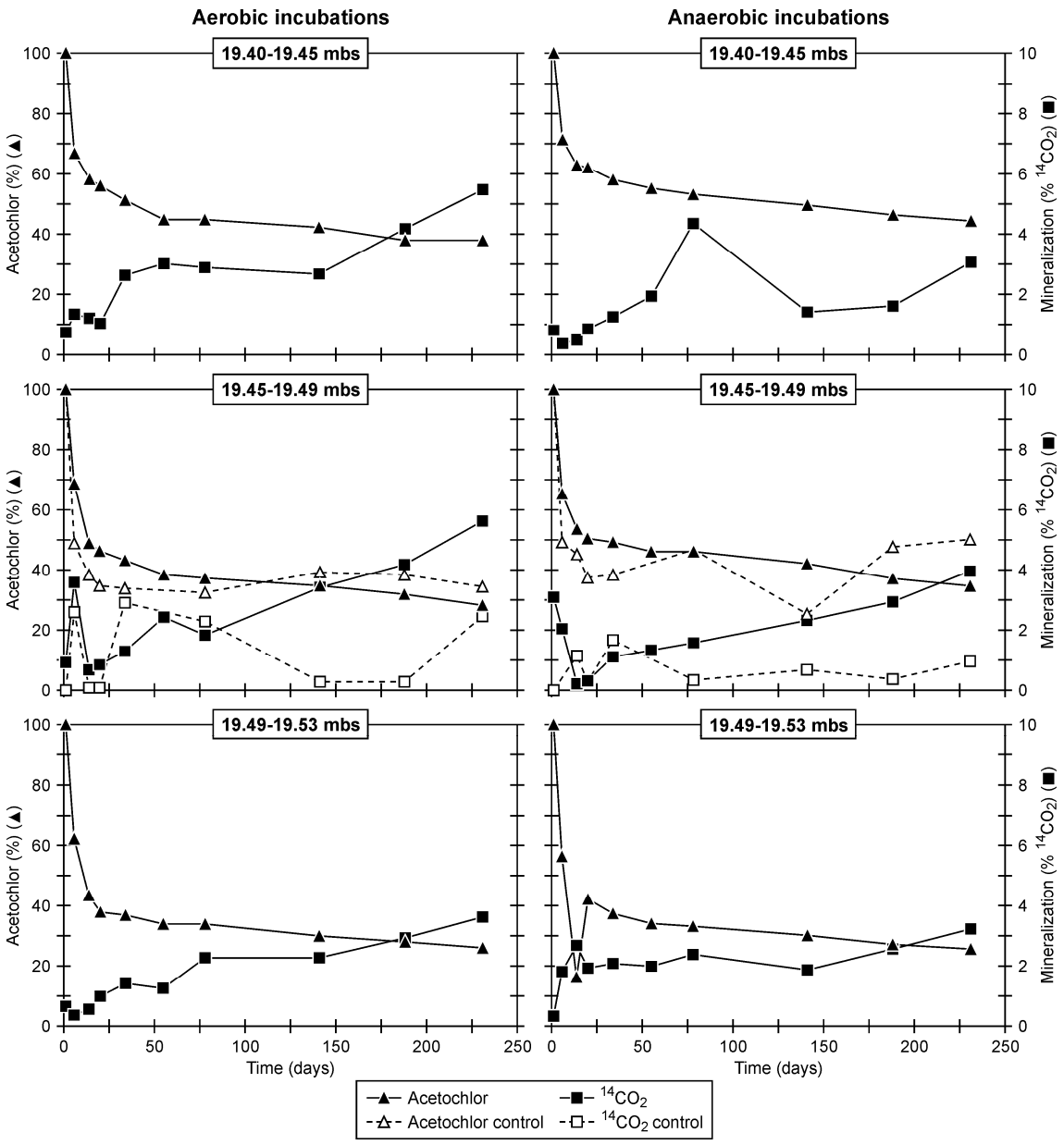


Figure 8: Acetochlor (^{14}C dissolved in water) and mineralized acetochlor (^{14}CO2) as a function of time (from Janniche et al., III).

4.5.3 Isoproturon

In most sandy aquifers isoproturon was not mineralized (table 5); not even degradation was observed in an aerobic continuous field injection experiment (Broholm et al., 2001). However, up to 40 % isoproturon incubated at 50 µg/L was mineralized in eight samples from both shallow and deep aerobic sandy aquifers with half-lifes from 28 days (after a 50 day lag period) to 3.6 years. Sediment from the deep aquifer was also capable of isoproturon mineralizing at a much lower and more realistic concentration (1 µg/L); though more limited (1.7-

4.7 %) and only in 3 of the 10 samples (Janniche et al., **I**). Pre-exposure did not enhance isoproturon degradation (Tuxen et al., 2002).

4.5.4 Mecoprop

Mecoprop is in general degraded and mineralized in aerobic sandy aquifers whereas degradation in anaerobic aquifers has not been reported (table 5). This was also observed in two field injection studies in shallow aquifers (about 5 mbs); in the aerobic aquifer 40 µg/L mecoprop was degraded to below 2 µg/L (Broholm et al., 2001) whereas in the anaerobic landfill leachate polluted aquifer no degradation was observed (injection concentration 150 µg/L) (Rugge et al., 1999). As the only one of the four herbicides, mecoprop is in some aquifer sediments easily mineralized with up to approximately 100 % mineralization within e.g. 22 days. Half-life ranged from 5 days to 9.5 years, though it was not degraded in every sample (table 5). There was a spatial variance in the mecoprop degradation potential within cm to few meters distance; also in pristine aquifers (Janniche et al., **II**; Klint et al., 1993; Mouvet et al., 2004; Tuxen et al., 2002). Furthermore, degradation rates were higher in pre-exposed aquifer sediments than in pristine sediment (Torang et al., 2003; Tuxen et al., 2002).

4.5.5 Summary on degradability in sandy aquifer

Degradation of acetochlor in sandy aquifers was only addressed in one study (Janniche et al., **III**), whereas the degradation of isoproturon, mecoprop and atrazine has often been studied, though without a clear understanding of when and how easily it takes place. In general:

- mecoprop was mineralized readily in aerobic sandy aquifers
- isoproturon may rarely be mineralized, but only slowly
- atrazine may be degradable only at concentrations in the mg-range
- acetochlor appears to be slowly mineralized in few selected samples from deep subsurface (19 mbs); hence the mineralization potential was not widespread.

Table 5: Degradation and mineralization in sandy aquifers

Herbicide	Depth (mbs)	N	Aerob/ Anaerob	C_o (ug/L)	C_o (ug/Kg)	T (days)	Temp (˚C)	Mineralization (%)	Degradation (%)	T½ (year)	Reference
Atrazine	1.7-1.9	2	Aerob	50	~75	~600	10	No	No	None	(Mouvet et al., 2004)
	2.0-3.0	1	Aerob	25	75	313	10	No	n.a.	None	(Larsen and Aamand, 2001)
	2-3[a]	1	Aerob	100-600	300-1800	74	~20	n.a.	No	None	(Agertved et al., 1992)
	2.5-5.8	6	Anaerob	50	~75	~600	10	No	No	None	(Mouvet et al., 2004)
	3-9	3	Anaerob	10 + 50	15 + 75	196	8-10	No	No	None	(Arildskov et al., 2001)
	3.4-5.0	6	Aerob	20-174	5.4	119	10	No (<0.5%)	n.a.	None	(Larsen et al., 2000)
	4.7-9.5	3	Anaerob	25	75	313	10	No	n.a.	None	(Larsen and Aamand, 2001)
	5.2-5.6	2	Aerob	4500 (d) 12000 (m)	23500 (d) 6000 (m)	23	25	No (<0.1)	Yes (<1.5)	3.5-4.0 (d)	(Mcmahon et al., 1992)
	5-6	1	Aerob	75-300	56-225	174	10	No	n.a.	None	(Klint et al., 1993)
	7.7	1	Anaerob	20-174	5.4	119	10	No (<0.5%)	n.a.	None	(Larsen et al., 2000)
	17.7-18.8	2	Anaerob	4500 (d) - 12000 (m)	23500 (d) - 6000 (m)	23	25	No (<0.1)	Yes (<1)	>42 (d)	(Mcmahon et al., 1992)
	18.4-47.1	6	Aerob	50	~75	~600	10	No	No	None	(Mouvet et al., 2004)
Isoproturon	1.7-1.9	2	Aerob	50	~75	~600	10	Yes	Yes	0.7-0.9 (d)	(Mouvet et al., 2004)
	2.0-3.0	1	Aerob	25	75	313	10	N.S. (<2.9)	n.a.	None	(Larsen and Aamand, 2001)
	2.5-5.8	6	Anaerob	50	~75	~600	10	No	No	None	(Mouvet et al., 2004)
	3.4-5.0	6	Aerob	14-123	3.8	267	10	N.S (<2)	n.a.	(~14-25)	(Larsen et al., 2000)
	4.7-9.5	3	Anaerob	25	75	313	10	N.S. (<1)	n.a.	None	(Larsen and Aamand, 2001)
	~5	5	Aerob	150	115[d]	120	10	n.a.	No	None	(Rugge et al., 1999)
	5-6	2	Aerob	25	-[b]	140	10	n.a.	No	None	(Tuxen et al., 2000)
	7.7	1	Anaerob	14-123	3.8	267	10	N.S. (<2)	n.a.	(~14-25)	(Larsen et al., 2000)
	10.5-58.5	7	Aerob	1	3	233	10	No	n.a.	None	(Janniche et al. l)
	19.25-19.4	3	Aerob	1	3	233	10	Yes (1.7-4.7)	n.a.	9.5-32	(Janniche et al. l)
	18.4-47.1	6	Aerob	50	~75	~600	10	Yes	Yes	0.08[c]-3.6 (d	(Mouvet et al., 2004)

Table 5 continued.

Herbicide	Depth (mbs)	N	Aerob/ Anaerob	C_o (ug/L)	C_o (ug/Kg)	T (days)	Temp (˚C)	Mineralization (%)	Degradation (%)	T½ (year)	Reference
Mecoprop	1.7-1.9	2	Aerob	50	~75	~600	10	Yes	Yes	0.07-5.8 (d)	(Mouvet et al., 2004)
	2.0-3.0	1	Aerob	25	75	313	10	6.6	n.a.	n.a.	(Larsen and Aamand, 2001)
	2-3[a]	1	Aerob	100-600	300-1800	74	~20	n.a.	No	None	(Agertved et al., 1992)
	2.5-5.8	6	Anaerob	50	~75	~600	10	No	No	None	(Mouvet et al., 2004)
	3	1	n.a.	0.10-10	0.10-10	308	10	9-11	n.a.	>4	(de Lipthay et al., 2007)
	3	1	n.a.	50-10000	50-10000	308	10	38-60	n.a.	0.7-1.9	(de Lipthay et al., 2007)
	3.4-5.0	6	Aerob	8-71	2.2	80	10	1.7-5	n.a.	1-7	(Larsen et al., 2000)
	4.6-7.9	3	Aerob	1	1.8[d]	341	10	n.a.	N.S.-90	<3	(Torang et al., 2003)
	4.6-7.9	3	Aerob	25	44[d]	341	10	1.3-83	n.a.	>0.02	(Torang et al., 2003)
	4.6-7.9	3	Aerob	100	175[d]	341	10	n.a.	<99	>0.1	(Torang et al., 2003)
	4.7-9.5	3	Anaerob	25	75	313	10	N.S. (1-3)	n.a.	None	(Larsen and Aamand, 2001)
	~5	5	Anaerob	150	115[d]	120	10	n.a.	No	None	(Rugge et al., 1999)
	5.0-6.0	7	Aerob	25	38[d]	180	10	Yes (<70)	Yes (<100)	n.a.	(Tuxen et al., 2002)
	5.0-6.0	3	Aerob	25	38[d]	180	10	No	No	None	(Tuxen et al., 2002)
	5-6	2	Aerob	25	-[b]	140	10	n.a.	Yes	0.01-0.03	(Tuxen et al., 2000)
	5-6	1	Aerob	75-300	56-225	22	10	~97-100	n.a.	n.a.	(Klint et al., 1993)
	7.7	1	Anaerob	8-71	2.2	80	10	N.S. (1.7)	n.a.	(7)	(Larsen et al., 2000)
	10.5-58.5	10	Aerob	1	3	233	10	4-28	n.a.	1.0-9.5	(Janniche et al., **I**)
	18.4-39.3	5	Aerob	50	~75	~600	10	Yes	Yes	0.03-0.3[e] (d)	(Mouvet et al., 2004)
	46.8-47.1	1	Aerob	50	~75	~600	10	No	No	None	(Mouvet et al., 2004)
Acetochlor	1.7-1.9	2	Aerob	50	~75	~600	10	n.a.	No	None	(Janniche et al., **III**)
	2.5-5.8	6	Anaerob	50	~75	~600	10	n.a.	No	None	(Janniche et al., **III**)
	10.5-58.5	5	Aerob	1	3	233	10	No	n.a.	None	(Janniche et al., **III**)
	18.4-47.1	6	Aerob	50	~75	~600	10	n.a.	No	None	(Janniche et al., **III**)
	19.25-19.53	5	Aerob	1	3	233	10	Yes (3.0-5.6)	n.a.	9.5-19	(Janniche et al., **III**)

N: number of environmental samples. C_0: initial concentration. T: incubation time. Temp: incubation temperature. $T_{½}$: half-life. (m): mineralization investigation. (d): degradation investigation. [a]: not clearly stated. [b]: column experiment. [c]: after a lag phase of 50 days. [d]: calculated based on wet wight as dry weight of sediment not given; hence actual concentrations are higher. [e]: calculated for T ≤119 days.

4.6 Concluding remarks on degradation

Herbicide pollution due to agriculture often results in concentration in the low µg-range in deep subsurface, and hence investigations should be conducted in this concentration level if the results are to be used for predicting herbicide degradation in-situ. But even degradation investigations on point-source contaminations e.g. due to landfills or spills should be followed at least until the herbicide concentrations are below 0.1 µg/L because they still posses a threat to the quality of the groundwater. E.g. in a degradation study of phenoxy acid herbicides in limestone aquifer, the concentrations decreased during 14 days of incubation to below the detection limit leaving less than 0.5 % equaling 10 µg/L (Harrison et al., 1998) which is still a high groundwater concentration.

Sorption only delays herbicide transport, whereas degradation removes the contaminant from the system. The transformation of herbicides in subsurface is mainly due to biological degradation that has to be complete (i.e. mineralization) to eliminate the pollution. Herbicide degradation in surface soils and shallow aquifers has often been investigated, whereas the investigations are more limited in deep subsurface (>10 mbs) and even more limited in carbonate rock systems. In general, atrazine, acetochlor, isoproturon, and mecoprop are all degradable, and they are even mineralizable in limestone/chalk and in sandy aquifers though degradation does not always take place. Whether or not an herbicide will be degraded is difficult to predict, but aerobic conditions still favors degradation more than anaerobic conditions. Mineralization at environmentally relevant low concentrations (<10 µg/L) was evident even in deep subsurface limestone and sandy aquifers of acetochlor, isoproturon and mecoprop, but not of atrazine (table 6). Although it was slow, it can still become significant considering the long residence time in the aquifer system.

Table 6: Half-lives determined at concentrations below 10 µg/L in deep subsurface (>10 mbs) samples.

Herbicide	Half-life[a] (year)	
	Limestone/ chalk	Sandy aquifer
Atrazine	None	n.i.
Mecoprop	9.5-none	1.0-none
Acetochlor	27-none	9.5-none
Isoproturon	95-none	9.5-none

[a]: based on mineralization. n.i.: not investigated.

5 Small-scale variations

Spatial variability in herbicide degradation and sorption have mainly been studied in topsoil or the upper meter of agricultural fields (Bending et al., 2001; Fredslund et al., 2008; Jacques et al., 1999; Vinther et al., 2008; Walker et al., 2001; Walker et al., 2002) and even at the millimeter scale (Gonod et al., 2003). As example, Wood et al. (2002) demonstrated evident small-scale variation in mineralization of atrazine and isoproturon in 10 cm discrete samples from 0.1-0.8 mbs; the mineralization rate decreased with increasing depths. The spatial variability in herbicide fate has also been studied in subsurface materials and aquifers. *Horizontal variability* in aquifers of herbicide degradation potential was reported within catchments (Heron and Christensen, 1992; Janniche et al. **IV;** Johnson et al., 2000). E.g. between samples taken with few meters distance to hundreds meters varied the degradation rate for 100 µg/L isoproturon in four groundwater samples 0-425 ng/day (Johnson et al., 2000). Furthermore, a *temporary variability* has also been reported (e.g. fast degradation one year but no degradation potential two-three years later) in the groundwater (Janniche et al., **IV**; Johnson et al., 2000). *Vertical variability* was reported for herbicide degradation, and the degradation was in general fast in samples from topsoil and down to 0.5 mbs, and limited in samples from >1 mbs (Charnay et al., 2005; Janniche et al. **I;** Larsen et al., 2000; Mouvet et al., 2004; Wood et al., 2002). Though the degradation rate decreased with depth, subsurface samples with increased degradation rates compared to the adjacent samples were evident. Sorption was investigated in mainly limestone profiles and although it in general decreased with depth, it was not correlated to depth, but to the clay or organic matter content (Coquet et al., 2004; Mouvet et al., 2004). *Vertical spatial variability at the cm-scale* in the subsurface has rarely been studied.

5.1 Limestone

Janniche et al. (**II**) found large variation in sorption within 10 limestone samples from four core sections taken within the same 4.5 km^2 catchment (figure 9). Mecoprop only had substantial sorption to one sample (2A), wheras isoproturon and acetochlor sorption was negligible in three of the sections (A, B and C), but marked in section D with a clear small-scale shift: the two uppermost samples (D1 and D2) had high sorption (isoproturon: 0.17-0.25 L/kg and acetochlor: 0.22-0.34 L/kg), and the samples below (D3 and D4) had lower sorption (isoproturon: 0.03-0.07 L/kg and acetochlor: <0.01-0.11 L/kg). The higher

specific surface area and lower TOC of D1 and D2 compared to the other limestone samples indicated that isoproturon and acetochlor sorption to a higher degree was controlled by mineralogy than by organic carbon content (Janniche et al., **II**).

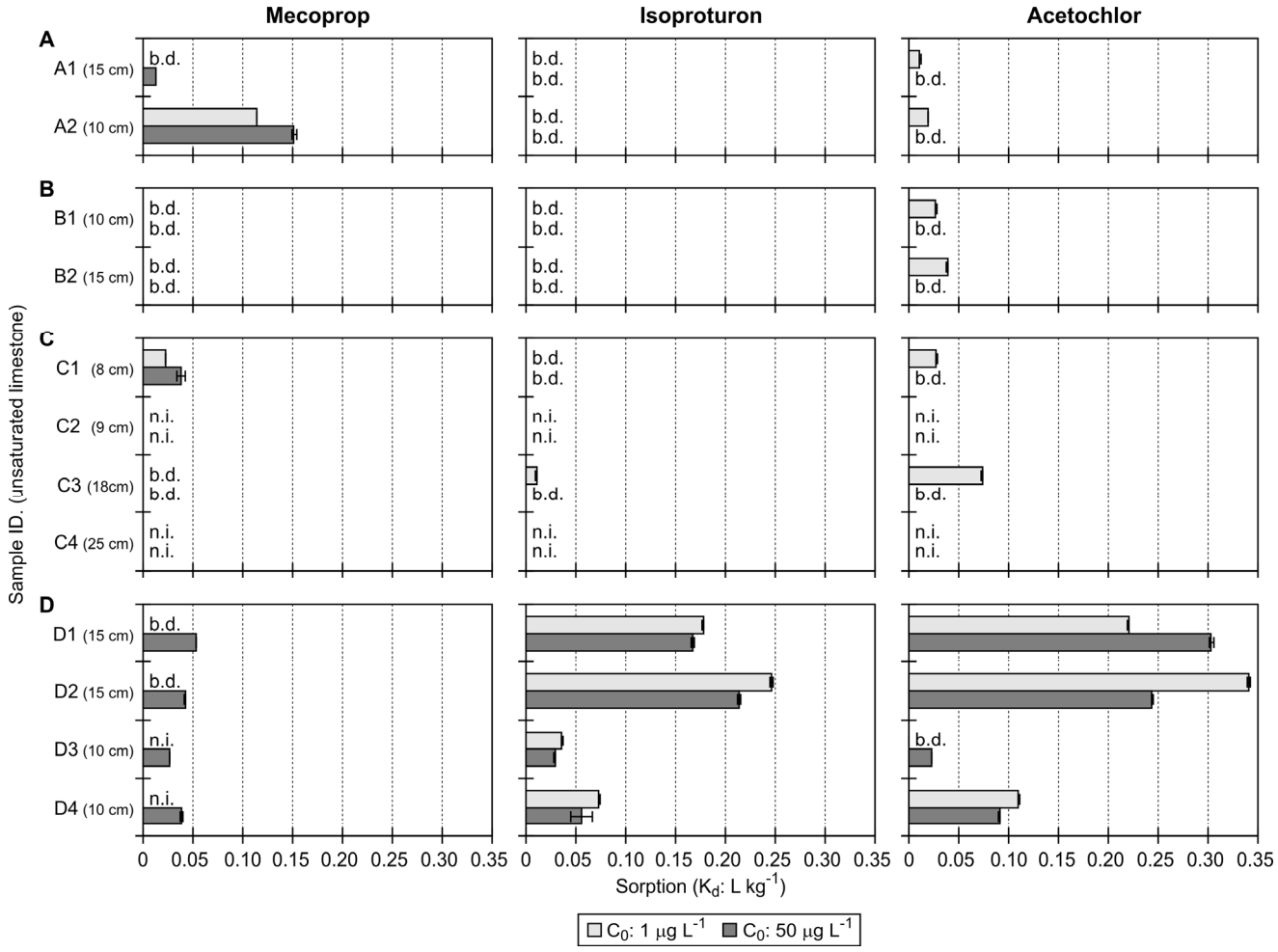


Figure 9: Sorption of mecoprop, isoproturon and acetochlor to 4 limestone sections. (Modified from Janniche et al., II)

The samples with high sorption also had fast mineralization (Janniche et al., **II**); hence the bioavailability of the herbicides was not hindered by the sorption. Already after 14 days of incubation the mineralization in D1 and D2 was noticeable higher than in D3 and D4 (Figure 10). Furthermore, the $^{14}CO_2$ evolution in D4 was at the same level as in autoclaved incubations. Hence, a clear small-scale shift in sorption and mineralization potential within only 25 cm was evident in the limestone.

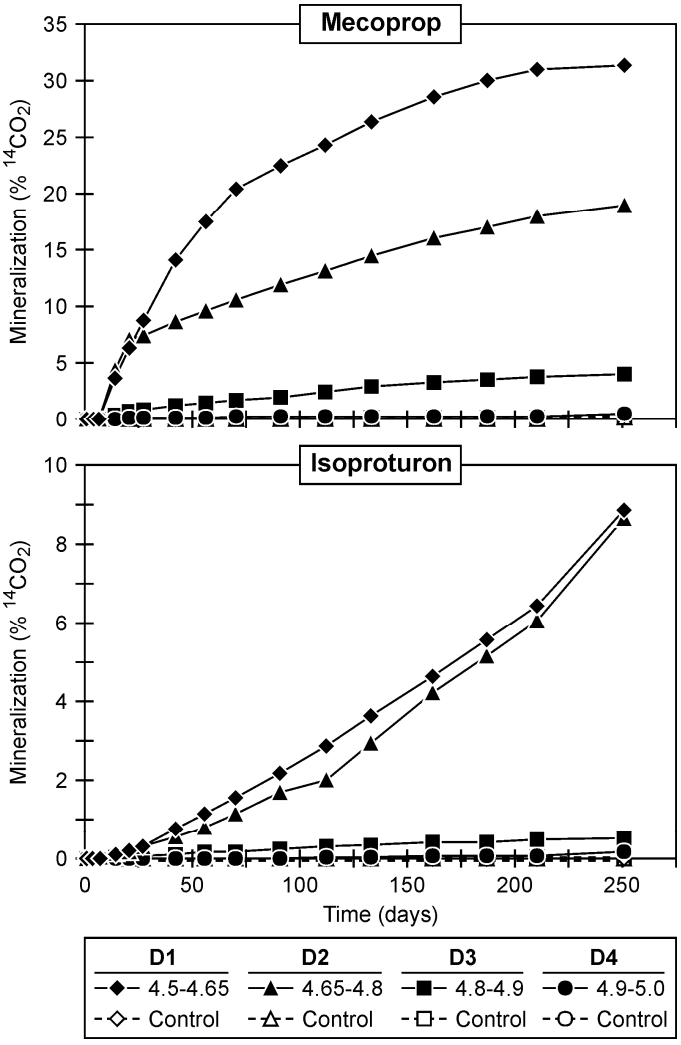


Figure 10: Small-scale variation in mecoprop and isoproturon mineralization as a function of time in 4 samples from an intact core (From Janniche et al., II).

5.2 Sandy aquifer

Small-scale variation in sorption was also observed by Janniche et al. (**II**) in an intact core section of sandy aquifer sediment divided horizontally into seven samples; a grayish color was observed in the bottom two subsamples of the aquifer section indicating reduced conditions. Mecoprop sorption was limited, whereas isoproturon and acetohclor sorption was low in the two uppermost samples, and increased gradually with depth (figure 11). Acetochlor sorption increased in average 78 % and isoproturon in average 61 % pr every five cm in the sandy aquifer section. The increase in K_d over depth could not be explained solely by variations in clay content or organic content, but was probably due to the reduced conditions in the bottom part of the core. Hence a substantial small-

scale variance in acetochlor and isoproturon sorption within these 33 cm was evident, and may be induced by changes in redox conditions.

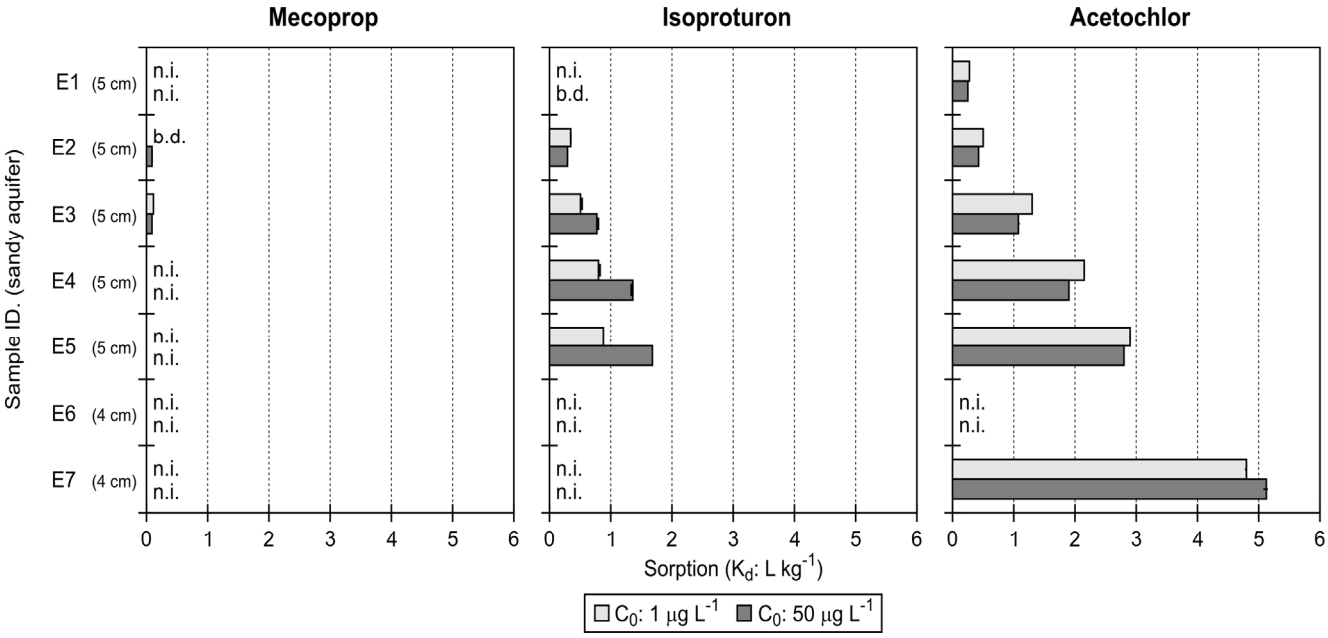


Figure 11: Sorption of mecoprop, isoproturon and acetochlor to a sandy aquifer column from Brévilles, France (Modified from Janniche et al., II).

Small-scale variation in herbicide mineralization was also evident in samples from a deep sandy aquifer (figure 12) (Janniche et al., **II**; Mouvet et al., 2004). A 20 cm long core dived over depth in five 4-cm samples, incubated with 50 µg/L mecoprop only had substantial mecoprop mineralization in the three bottommost samples with 34-72% mineralized (figure 12A) and 56-97 % degraded in about 500 days (Mouvet et al., 2004). In another study with sediment from the same aquifer isoproturon, mecoprop and acetochlor mineralization was investigated at 1 µg/l in a section where acetochlor and isoproturon sorption increased with depth (figure 12B) (Janniche et al., **II**). Mecoprop was mineralized (11-22 %) in all six aquifer samples during 330 days, whereas isoproturon was only mineralized (2-5 %) in the three uppermost samples, and acetochlor was mineralized (3-6 %) in every sample except in the second uppermost (figure 12B). Hence, in this sediment the small-scale variation in mecoprop mineralization was less distinct than in the study by Mouvet et al. (2004). Nevertheless, there was clearly small-scale variation in isoproturon mineralization.

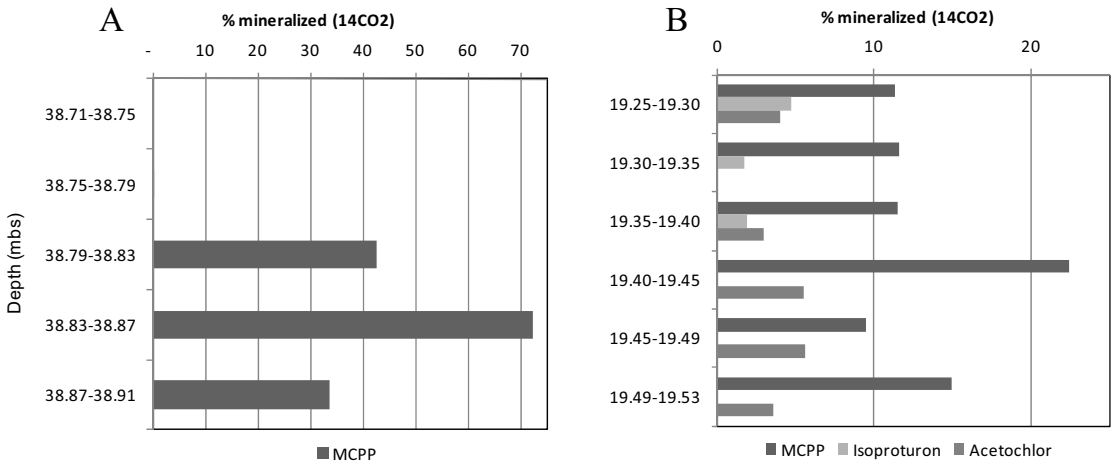


Figure 12: Vertical small-scale variation in herbicide mineralization in sandy aquifer from Brévilles. A) Based on Mouvet et al., 2004. B) Modified from Janniche et al., II.

5.3 Implications of small-scale variations

There is a spatial variability in herbicide degradation potential even in small catchments, and also a temporary variability, meaning that a degradation result obtained based on sampling from one specific day may not be transistent with results in the years to come and results from specific samples within a field site may not be representative of the whole site. This opens up for a discussion of whether the samples and results obtained are representative – and what these results can be used for. Can they be used in fate modelling? Or can they only be used to say that there has been observed a potential for degradation of herbicides, but it is unknown how the potential is now? The fact that there is both spatial and temporal variability reflects that the system is very complex.

Some of the major parameters in modelling the fate of pollutants in the environment are degradation and sorption because these parameters dominate Natural Attenuation processes. It is therefore crucial not only to know the geology and hydrology of a site but also the degradation and sorption. If sediment layers with increased mineralization could be identified, it would be a step towards better understanding the interaction between sediment and biodegradation which may improve the predictions and modeling of the fate. Laboratory investigation with sediment samples is one method to determine sorption and degradation – but how close should the sediment samples be sampled? What scale should one work with?

5.3.1 Fate evaluated with small-scale variations

Spatial small-scale variations seen as subsurface zones of increased mineralization potential are evident in figure 13, where two distinct active zones are present: one in the unsaturated limestone around 5 mbs and one in the sandy aquifer around 20 mbs – both in the core Pz17c (Jannichet et al., **I**).

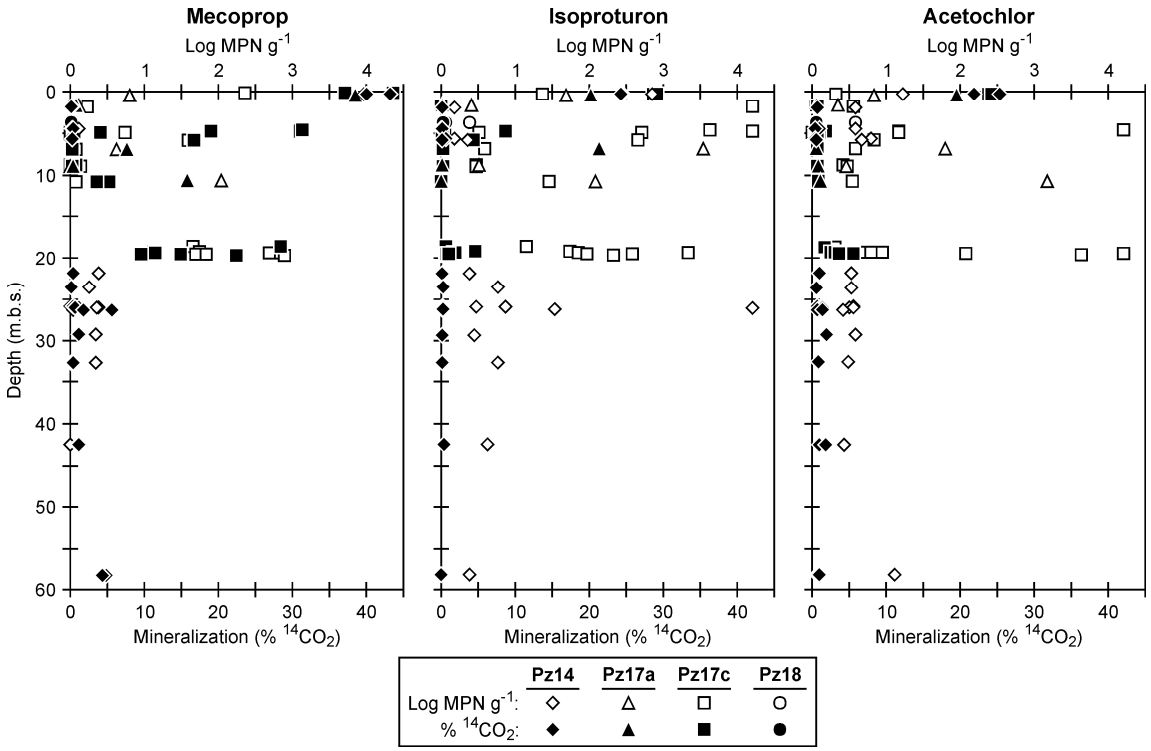
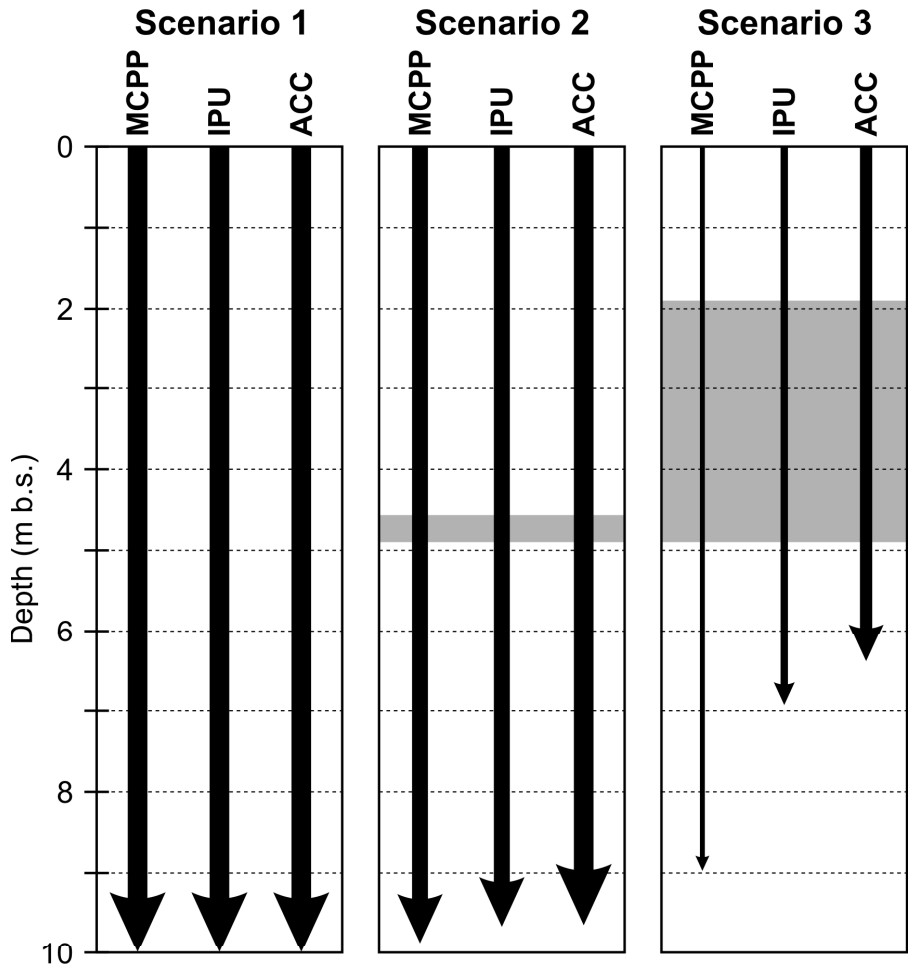


Figure 13: Number of specific degraders (log MPN g^{-1}) and mineralization (% $^{14}CO_2$) of mecoprop, isoproturon and acetochlor as a function of depth in the cores. M.b.s: meter below surface. Groundwater level was 44.28 mbs in Pz14, 9.58 mbs in Pz17a, 9.39 mbs in Pz17c and not present in Pz18.

To evaluate the importance of such an active layer with increased sorption and mineralization within the unsaturated limestone, three scenarios were set up for the fate of mecoprop, isoproturon and acetochlor in a 10 m thick unsaturated limestone: 1) no active layer, 2) a 30 cm active layer, and 3) a 3 m active layer (figure 14). Sorption coefficients and mineralization rates in the active layer were based on actually measured values (Janniche et al., **II**) and neglected in the "non-active" limestone. The 1 m/year water flow velocity (v_w) calculated earlier for this catchment was used (Gutierrez and Baran, 2009). Retardation due to sorption (delay) was calculated as: $(x_1\ \rho_b\ K_d)/(v_w\ \varepsilon)$, where x_1 is the thickness of active layer, ρ_b is bulk density set to 2 kg/L, K_d is the sorption coefficient determined for each herbicide, and ε is the porosity set to 0.4. Removal due to mineralization

was calculated as: $\exp[(k\ x_1/v_w)(1+\ \rho_b\ K_d/\varepsilon)]$ where k is the mineralization rate determined for each herbicide. In scenario 1 there is no mineralization or sorption, hence the herbicides are transported through the limestone to the groundwater in 10 years. In scenario 2 the presence of a 30 cm thick layer will lead to only a very limited Natural Attenuation. Depending on the herbicide 1-3 % would be mineralized, and it would take the herbicides 0.1-0.4 years longer to reach the groundwater (figure 14). However, if the total extent of active layers was 3 m over the whole 10 m profile, then the breakthrough times would be 10.9 years (mecoprop), 13 years (isoproturon) and 13.6 years (acetochlor) respectively. The proportion of mineralized herbicide would then become 39 % mecoprop, 33 % isoproturon and only 8 % acetochlor.

Conclusively, if the active layer is only 30 cm thick, then the Natural Attenuation due to sorption and mineralization will be negligible, whereas the effect will be notable if the layer is 10 times thicker. Considering that the unsaturated limestone in the investigated field site is up to 42 m thick it is not unlikely that the summed extend of an active layer will be greater than the observed 30 cm. Hence, the importance of such active layers in the unsaturated limestone depends on their magnitude, and consequently a very detailed knowledge of the limestone characteristics of the site is paramount.



	MCPP			IPU			ACC		
K_d (L kg^{-1})	0.06			0.2			0.24		
$T_{½}$ (y)	1.3			4.6			23.8		
	S1	**S2**	**S3**	**S1**	**S2**	**S3**	**S1**	**S2**	**S3**
Delayed (y)	0.0	0.1	0.9	0.0	0.3	3.0	0.0	0.4	3.6
Removed (%)	0	5	39	0	3	33	0	1	8

Figure 14: Three fate scenarios of herbicides mecoprop (MCPP), isoproturon (IPU) and acetochlor (ACC) through a 10 m thick limestone profile: Scenario 1 (S1): no active layer, Scenario 2 (S2): 0.30 m active layer, Scenario 3 (S3): 3 m active layer. The length of arrows indicates the delay in herbicide transport due to sorption (based on linear sorption coefficient: K_d) in active layers, and the width of arrows reflects the residual mass of herbicides due to mineralization (based on half-lifes: $T_{½}$).

6 Conclusions

Samples of limestone, sandy aquifer sediment, and groundwater collected down to 59 mbs were investigated for sorption and mineralization of isoproturon, acetochlor, atrazine, and mecoprop at low concentrations. The conclusions can be summarized to:

- **Sorption to limestone** and chalk was in general limited (K_d ≤0.55 L/kg) and decreased, depending on the herbicide, in the following order: isoproturon>acetochlor>atrazine>mecoprop. Thus, these geological settings provide only limited protection from groundwater pollution.
- **Sorption to sandy aquifers** increased in the order mecoprop<atrazine< isoproturon<acetochlor, and was highest in anaerobic sediments. While mecoprop sorption was limited (<0.3 L/kg), atrazine K_d-values were up to 1.9 L/kg, and substantial isoproturon (≤3.1 L/kg) and acetochlor sorption was evident (≤5.1 L/kg). Retardation of isoproturon and acetochlor due to sorption can therefore be considerable in sandy aquifers.
- **Degradation** and even mineralization in deep (>10 mbs) subsurface **limestone and chalk** at <10 µg/L was observed for mecoprop, acetochlor and isoproturon, although it was slow (half-life ≥9.5 years).
- **Degradation in sandy aquifer** (>10 mbs and <10 µg/L) was only investigated for mecoprop, acetochlor and isoproturon, which were mineralized slowly, though with shorter half-lives (≥1.0 year) than observed in deep subsurface limestone and chalk.
- **Spatial variability** in sorption and degradation was evident even within layers of few cm thicknesses, and the implications on an herbicide's fate in a catchment depend on the total magnitude of the layers with increased sorption or degradation.

Recommendations

It is difficult to predict if an herbicide will be degraded/mineralized in a specific environment because of the great spatial variability, and the degradability might not be constant over time. This variability should be included when modeling the fate of herbicides in a catchment to provide better and more precise predictions. To consider the spatial variability other sampling strategies should be included than just the more conventional random sampling. Sampling should be focused on changes in structures to ensure that especially samples from around an even minor shift in geology or in color are included since there can be major

differences in both sorption and mineralization potential within just a few cm. Furthermore, sampling in hard rock should include fractures since bacteria mainly are found adhered to fracture walls and not in matrix.

References

Agertved, J., Rugge, K., Barker, J.F., 1992. Transformation of the Herbicides Mcpp and Atrazine Under Natural Aquifer Conditions. Ground Water, 30(4): 500-506.

Albrechtsen, H.-J., Mills, M.S., Aamand, J., Bjerg, P.L., 2001. Degradation of herbicides in shallow Danish aquifers: an integrated laboratory and field study. Pest Management Science, 57(4): 341-350.

Albrechtsen, H.-J., Smith, P.M., Nielsen, P., Christensen, T.H., 1996. Significance of biomass support particles in laboratory studies on microbial degradation of organic chemicals in aquifers. Water Research, 30(12): 2977-2984.

Albrechtsen, H.-J., Smith, P.M., Nielsen, P., Christensen, T.H., 1997. Importance of sediment fines in laboratory studies on the degradation of organic chemicals in aquifers. Water Research, 31(9): 2287-2299.

Alexander, M., 1999. Biodegradation and Bioremediation. Academic Press, San Diego, California.

Alletto, L., Coquet, Y., Benoit, P., Bergheaud, V., 2006. Effects of temperature and water content on degradation of isoproturon in three soil profiles. Chemosphere, 64(7): 1053-1061.

Arias-Estevez, M. et al., 2008. The mobility and degradation of pesticides in soils and the pollution of groundwater resources. Agriculture Ecosystems & Environment, 123(4): 247-260.

Arildskov, N.P., Pedersen, P.G., Albrechtsen, H.-J., 2001. Fate of the herbicides 2,4,5-t, atrazine, and DNOC in a shallow, anaerobic aquifer investigated by in situ passive diffusive emitters and laboratory batch experiments. Ground Water, 39(6): 819-830.

Baran, N., Mouvet, C., Dagnac, T., Jeannot, R., 2004. Infiltration of acetochlor and two of its metabolites in two contrasting soils. Journal of Environmental Quality, 33(1): 241-249.

Baran, N., Mouvet, C., Negrel, P., 2007. Hydrodynamic and geochemical constraints on pesticide concentrations in the groundwater of an agricultural catchment (Brevilles, France). Environmental Pollution, 148(3): 729-738.

Battaglin, W.A., Furlong, E.T., Burkhardt, M.R., Peter, C.J., 2000. Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998. Science of the Total Environment, 248(2-3): 123-133.

Bending, G.D., Rodriguez-Cruz, M.S., 2007. Microbial aspects of the interaction between soil depth and biodegradation of the herbicide isoproturon. Chemosphere, 66(4): 664-671.

Bending, G.D., Shaw, E., Walker, A., 2001. Spatial heterogeneity in the metabolism and dynamics of isoproturon degrading microbial communities in soil. Biology and Fertility of Soils, 33(6): 484-489.

Besien, T.J., Williams, R.J., Johnson, A.C., 2000. The transport and behaviour of isoproturon in unsaturated chalk cores. Journal of Contaminant Hydrology, 43(2): 91-110.

Boethling, R.S., Alexander, M., 1979. Effect of Concentration of Organic Chemicals on Their Biodegradation by Natural Microbial Communities. Applied and Environmental Microbiology, 37(6): 1211-1216.

Boyd, E.S., Cummings, D.E., Geesey, G.G., 2007. Mineralogy influences structure and diversity of ba communities associated with geological substrata in a pristine aquifer. Microbial Ecology, 54(1): 170-182.

Boyd, R.A., 2000. Herbicides and herbicide degradates in shallow groundwater and the Cedar River near a municipal well field, Cedar Rapids, Iowa. Science of the Total Environment, 248(2-3): 241-253.

Broholm, M.M. et al., 2001. Fate of herbicides in a shallow aerobic aquifer: A continuous field injection experiment (Vejen, Denmark). Water Resources Research, 37(12): 3163-3176.

Buss, S.R., Thrasher, J., Morgan, P., Smith, J.W.N., 2006. A review of mecoprop attenuation in the subsurface. Quarterly Journal of Engineering Geology and Hydrogeology, 39: 283-292.

Charnay, M.P., Tuis, S., Coquet, Y., Barriuso, E., 2005. Spatial variability in C-14-herbicide degradation in surface and subsurface soils. Pest Management Science, 61(9): 845-855.

Chilton, P.J., Stuart, M.E., Gooddy, D.C., Williams, R.J., Johnson, A.C., 2005. Pesticide fate and behaviour in the UK Chalk aquifer, and implications for groundwater quality. Quarterly Journal of Engineering Geology and Hydrogeology, 38: 65-81.

Clausen, L., Fabricius, I., 2001. Atrazine, isoproturon, mecoprop, 2,4-D, and bentazone adsorption onto iron oxides. Journal of Environmental Quality, 30(3): 858-869.

Clausen, L., Fabricius, I., Madsen, L., 2001. Adsorption of pesticides onto quartz, calcite, kaolinite, and alpha-alumina. Journal of Environmental Quality, 30(3): 846-857.

Clausen, L., Larsen, F., Albrechtsen, H.-J., 2004. Sorption of the herbicide dichlobenil and the metabolite 2,6-dichlorobenzamide on soils and aquifer sediments. Environmental Science & Technology, 38(17): 4510-4518.

Coquet, Y., Ribiere, C., Vachier, P., 2004. Pesticide adsorption in the vadose zone: a case study on Eocene and Quaternary materials in Northern France. Pest Management Science, 60(10): 992-1000.

de Guzman, N.P. et al., 2005. The acetochlor registration partnership state ground water monitoring program. Journal of Environmental Quality, 34(3): 793-803.

de Lipthay, J.R., Sorensen, S.R., Aamand, J., 2007. Effect of herbicide concentration and organic and inorganic nutrient amendment on the mineralization of mecoprop, 2,4-D and 2,4,5-T in soil and aquifer samples. Environmental Pollution, 148(1): 83-93.

Dictor, M.C., Baran, N., Gautier, A., Mouvet, C., 2008. Acetochlor mineralization and fate of its two major metabolites in two soils under laboratory conditions. Chemosphere, 71(4): 663-670.

European Parliament and Council, 2006. Directive 2006/118/EC of the European Parliament and of the Council of 12 December 2006 on the protection of groundwater against pollution and deterioration.

Fetter, C. W., 2001. Applied Hydrogeology. Prentice Hall, Upper Saddle River, N.J.

Fomsgaard, I.S., 1995. Degradation of Pesticides in Subsurface Soils, Unsaturated Zone - A Review of Methods and Results. International Journal of Environmental Analytical Chemistry, 58(1-4): 231-245.

Formsgaard, I. S., 2004. The influence of Sorption on the Degradation of Pesticides and other Chemicals in Soil. 902 Danish Environmental Protection Agency,

Fracflow, 2001. Contaminat transport, monitoring techniques, and remediation strategies in cross European fractured chalk (Final Report). Contract No.: ENV4-CT97-0441

Fredslund, L. et al., 2008. Spatial variation in 2-methyl-4-chlorophenoxyacetic acid mineralization and sorption in a sandy soil at field. Journal of Environmental Quality, 37(5): 1918-1928.

GEUS, 2007. Grundvand, Status og udvikling 1989 - 2006. www.grundvandsovervaagning.dk.

GEUS, 2009. Grundvandsovervågning 2008. Grundvand, Status og udvikling 1989 - 2007. www.grundvandsovervaagning.dk [in Danish].

Goldscheider, N., Hunkeler, D., Rossi, P., 2006. Review: Microbial biocenoses in pristine aquifers and an assessment of investigative methods. Hydrogeology Journal, 14(6): 926-941.

Gonod, L.V., Chenu, C., Soulas, G., 2003. Spatial variability of 2,4-dichlorophenoxyacetic acid (2,4-D) mineralisation potential at a millimetre scale in soil. Soil Biology & Biochemistry, 35(3): 373-382.

Graber, E.R., Bonisover, M., 2003. Competitive sorption of organic contaminants in chalk. Journal of Contaminant Hydrology, 67(1-4): 159-175.

Grathwohl, P., 1990. Influence of Organic-Matter from Soils and Sediments from Various Origins on the Sorption of Some Chlorinated Aliphatic-Hydrocarbons - Implications on Koc Correlations. Environmental Science & Technology, 24(11): 1687-1693.

Griebler, C., Lueders, T., 2009. Microbial biodiversity in groundwater ecosystems. Freshwater Biology, 54(4): 649-677.

Hallberg, G.R., 1989. Pesticide Pollution of Groundwater in the Humid United-States. Agriculture Ecosystems & Environment, 26(3-4): 299-367.

Haria, A.H., Hodnett, M.G., Johnson, A.C., 2003. Mechanisms of groundwater recharge and pesticide penetration to a chalk aquifer in southern England. Journal of Hydrology, 275(1-2): 122-137.

Harrison, I., Leader, R.U., Higgo, J.J.W., Williams, G.M., 1998. A study of the degradation of phenoxy acid herbicides at different sites in a limestone aquifer. Chemosphere, 36(6): 1211-1232.

Helweg, A., Fomsgaard, I.S., Reffstrup, T.K., Sorensen, H., 1998. Degradation of mecoprop and isoproturon in soil influence of initial concentration. International Journal of Environmental Analytical Chemistry, 70(1-4): 133-148.

Heron, G., Christensen, T.H., 1992. Degradation of the Herbicide Mecoprop in An Aerobic Aquifer Determined by Laboratory Batch Studies. Chemosphere, 24(5): 547-557.

Hiller, E., Krascsenits, Z., Cernansky, S., 2008. Sorption of acetochlor, atrazine, 2,4-D, chlorotoluron, MCPA, and trifluralin in six soils from Slovakia. Bulletin of Environmental Contamination and Toxicology, 80(5): 412-416.

Holm, P.E., Nielsen, P.H., Albrechtsen, H.-J., Christensen, T.H., 1992. Importance of Unattached Bacteria and Bacteria Attached to Sediment in Determining Potentials for Degradation of Xenobiotic Organic

Contaminants in An Aerobic Aquifer. Applied and Environmental Microbiology, 58(9): 3020-3026.

Issa, S., Wood, M., 1999. Degradation of atrazine and isoproturon in the unsaturated zone: a study from Southern England. Pesticide Science, 55(5): 539-545.

Issa, S., Wood, M., 2005. Degradation of atrazine and isoproturon in surface and sub-surface soil materials undergoing different moisture and aeration conditions. Pest Management Science, 61(2): 126-132.

Jacques, D., Mouvet, C., Mohanty, B., Vereecken, H., Feyen, J., 1999. Spatial variability of atrazine sorption parameters and other soil properties in a podzoluvisol. Journal of Contaminant Hydrology, 36(1-2): 31-52.

Johnson, A.C., Hughes, C.D., Williams, R.J., Chilton, P.J., 1998. Potential for aerobic isoproturon biodegradation and sorption in the unsaturated and saturated zones of a chalk aquifer. Journal of Contaminant Hydrology, 30(3-4): 281-297.

Johnson, A.C., White, C., Bhardwaj, C.L., 2000. Potential for isoproturon, atrazine and mecoprop to be degraded within a chalk aquifer system. Journal of Contaminant Hydrology, 44(1): 1-18.

Johnson, A.C., White, C., Bhardwaj, C.L., Dixon, A., 2003. The ability of indigenous micro-organisms to degrade isoproturon, atrazine and mecoprop within aerobic UK aquifer systems. Pest Management Science, 59(12): 1291-1302.

Kalkhoff, S.J., Kolpin, D.W., Thurman, E.M., Ferrer, I., Barcelo, D., 1998. Degradation of chloroacetanilide herbicides: The prevalence of sulfonic and oxanilic acid metabolites in Iowa groundwaters and surface waters. Environmental Science & Technology, 32(11): 1738-1740.

Klint, M., Arvin, E., Jensen, B.K., 1993. Degradation of the pesticides Mecoprop and Atrazine in unpolluted sandy aquifers. Journal of Environmental Quality, 22: 262-266.

Kolpin, D.W., Thurman, E.M., Linhart, S.M., 1998. The environmental occurrence of herbicides: The importance of degradates in ground water.

Archives of Environmental Contamination and Toxicology, 35(3): 385-390.

Kolpin, D.W., Kalkhoff, S.J., Goolsby, D.A., Sneck-Fahrer, D.A., Thurman, E.M., 1997. Occurrence of selected herbicides and herbicide degradation products in Iowa's ground water, 1995. Ground Water, 35(4): 679-688.

Kristensen, G.B., Johannesen, H., Aamand, J., 2001a. Mineralization of aged atrazine and mecoprop in soil and aquifer chalk. Chemosphere, 45(6-7): 927-934.

Kristensen, G.B., Sorensen, S.R., Aamand, J., 2001b. Mineralization of 2,4-D, mecoprop, isoproturon and terbuthylazine in a chalk aquifer. Pest Management Science, 57(6): 531-536.

Larsen, G. et al., 1995. Vejledning i Ingeniørgeologisk prøvebeskrivelse (A guide to engineering geological soil description). Dansk Geoteknisk Forening (Danish Geotechnical Society),

Larsen, L., Aamand, J., 2001. Degradation of herbicides in two sandy aquifers under different redox conditions. Chemosphere, 44(2): 231-236.

Larsen, L., Sorensen, S.R., Aamand, J., 2000. Mecoprop, isoproturon, and atrazine in and above a sandy aquifer: Vertical distribution of mineralization potential. Environmental Science & Technology, 34(12): 2426-2430.

Loor-Vela, S.X., Simmons, J.J.C., Simmons, F.W., Raskin, L., 2003. Dissipation of [C-14]acetochlor herbicide under anaerobic aquatic conditions in flooded soil microcosms. Journal of Agricultural and Food Chemistry, 51(23): 6767-6773.

Madsen, L., Lindhardt, B., Rosenberg, P., Clausen, L., Fabricius, I., 2000. Pesticide sorption by low organic carbon sediments: a screening for seven herbicides. Journal of Environmental Quality, 29(5): 1488-1500.

Mathias, S.A., Butler, A.P., McIntyre, N., Wheater, H.S., 2005. The significance of flow in the matrix of the Chalk unsaturated zone. Journal of Hydrology, 310(1-4): 62-77.

Mcmahon, P.B., Chapelle, F.H., Jagucki, M.L., 1992. Atrazine Mineralization Potential of Alluvial-Aquifer Sediments Under Aerobic Conditions. Environmental Science & Technology, 26(8): 1556-1559.

Mills, M.S. et al., 2001. Quantification of acetochlor degradation in the unsaturated zone using two novel in situ field techniques: comparisons with laboratory-generated data and implications for groundwater risk assessments. Pest Management Science, 57(4): 351-359.

Moreau, C., Mouvet, C., 1997. Sorption and desorption of atrazine, deethylatrazine, and hydroxyatrazine by soil and aquifer solids. Journal of Environmental Quality, 26(2): 416-424.

Mouvet, C. et al., 2004. Pesticides in European groundwaters: Detailed study of representative aquifers and simulation of possible evolution scenarios PEGASE: Final report. BRGM,

Nadin, P., 2007. The use of plant protection products in the European Union, Data 1992-2003. Eurostat, Statistical books, 2007 edition , European Commission, Luxembourg.

OECD, 1993. OECD guidelines for testing of chemicals.106 Adsorption/desorption Paris, France.

Papiernik, S.K., Spalding, R.F., 1998. Atrazine, deethylatrazine, and deisopropylatrazine persistence measured in groundwater in situ under low-oxygen conditions. Journal of Agricultural and Food Chemistry, 46(2): 749-754.

Payaperez, A.B., Cortes, A., Sala, M.N., Larsen, B., 1992. Organic-Matter Fractions Controlling the Sorption of Atrazine in Sandy Soils. Chemosphere, 25(6): 887-898.

Poeton, T.S., Stensel, H.D., Strand, S.E., 1999. Biodegradation of polyaromatic hydrocarbons by marine bacteria: Effect of solid phase on degradation kinetics. Water Research, 33(3): 868-880.

Rae, J.E., Cooper, C.S., Parker, A., Peters, A., 1998. Pesticide sorption onto aquifer sediments. Journal of Geochemical Exploration, 64(1-3): 263-276.

Reffstrup, T.K., Sorensen, H., Helweg, A., 1998. Degradation of mecoprop at different concentrations in surface and sub-surface soil. Pesticide Science, 52(2): 126-132.

Roulier et al., 2006. Controls on atrazine leaching through a soil-unsaturated fractured limestone sequence at Brevilles, France. Journal of Contaminant Hydrology, 84(1-2): 81-105.

Rugge, K., Bay, H., Chrintz, T., Brüsch, W., Rosenberg, P., 2005. Pesticider i dansk grundvand: GRUMO- og Boringskontroldata. 1033 Miljøstyrelsen,

Rugge, K., Bjerg, P.L., Mosbaek, H., Christensen, T.H., 1999. Fate of MCPP and atrazine in an anaerobic landfill leachate plume (Grindsted, Denmark). Water Research, 33(10): 2455-2458.

Scheidleder J, et al., 1999. Groundwater quality and quantity in Europe. Environmental Assessment Report No. 3. European Environment Agency,

Sonnenborg, T. O., 2006. Vandressource- og stoftransport-modellering i kalk: status og muligheder. ATV,

Sorensen, S.R., Bending, G.D., Jacobsen, C.S., Walker, A., Aamand, J., 2003. Microbial degradation of isoproturon and related phenylurea herbicides in and below agricultural fields. Fems Microbiology Ecology, 45(1): 1-11.

Spliid, N.H., Koppen, B., 1998. Occurrence of pesticides in Danish shallow ground water. Chemosphere, 37(7): 1307-1316.

Taylor, J.P., Mills, M.S., Burns, R.G., 2005. Dissipation of acetochlor and its distribution in surface and sub-surface soil fractions during laboratory incubations. Pest Management Science, 61(6): 539-548.

The Commission of the European Communities, 2004. COMMISSION DECISION of 10 March 2004 concerning the non-inclusion of atrazine in Annex I to Council Directive 91/414/EEC and the withdrawal of authorisations for plant protection products containing this active substance. Official Journal of the European Union

Tomlin, C. D. S. e., 1997. The Pesticide Manual. British Crop Protection Concil, UK.

Torang, L., Nyholm, N., Albrechtsen, H.-J., 2003. Shifts in biodegradation kinetics of the herbicides MCPP and 2,4-D at low concentrations in aerobic aquifer materials. Environmental Science & Technology, 37(14): 3095-3103.

Turner, J. et al., 2006. Future trends in transport and fate of diffuse contaminants in catchments, with special emphasis on stable isotope applications. Hydrological Processes, 20(1): 205-213.

Tuxen, N., de Lipthay, J.R., Albrechtsen, H.-J., Aamand, J., Bjerg, P.L., 2002. Effect of exposure history on microbial herbicide degradation in an aerobic aquifer affected by a point source. Environmental Science & Technology, 36(10): 2205-2212.

Tuxen, N., Tuchsen, P.L., Rugge, K., Albrechtsen, H.-J., Bjerg, P.L., 2000. Fate of seven pesticides in an aerobic aquifer studied in column experiments. Chemosphere, 41(9): 1485-1494.

Vinther, F.P. et al., 2008. Field-scale variation in microbial activity and soil properties in relation to mineralization and sorption of pesticides in a sandy soil. Journal of Environmental Quality, 37(5): 1710-1718.

Walker, A., Bromilow, R.H., Nicholls, P.H., Evans, A.A., Smith, V.J.R., 2002. Spatial variability in the degradation rates of isoproturon and chlorotoluron in a clay soil. Weed Research, 42(1): 39-44.

Walker, A., Jurado-Exposito, M., Bending, G.D., Smith, V.J.R., 2001. Spatial variability in the degradation rate of isoproturon in soil. Environmental Pollution, 111(3): 407-415.

Wefer-Roehl, A., Graber, E.R., Adar, E., 2002. Retardation of Organic Contaminants in Natural Fractures in Chalk. Ground Water, 40(3): 291-301.

Wefer-Roehl, A. et al., 2001. Sorption of organic contaminants in a fractured chalk formation. Chemosphere, 44(5): 1121-1130.

Wellings, S.R., 1984. Recharge of the Upper Chalk Aquifer at A Site in Hampshire, England .1. Water-Balance and Unsaturated Flow. Journal of Hydrology, 69(1-4): 259-273.

Widmer, S.K., Spalding, R.F., 1995. A Natural Gradient Transport Study of Selected Herbicides. Journal of Environmental Quality, 24(3): 445-453.

Wood, M., Issa, S., Albuquerque, M., Johnson, A.C., 2002. Spatial variability in herbicide degradation in the subsurface environment of a groundwater protection zone. Pest Management Science, 58(1): 3-9.

Appendices

I. Janniche, G., Lindberg, E., Mouvet, C., Albrechtsen, H.-J. Distribution of isoproturon, mecoprop and acetochlor mineralization and mineralizers in deep unsaturated limestone and sandy aquifer, submitted to *Chemosphere*.

II. Janniche, G., Mouvet, C., Albrechtsen, H.-J. Vertical small-scale variations of sorption and mineralization of three herbicides in subsurface limestone and sandy aquifer, submitted to *Journal of Contaminant Hydrology*.

III. Janniche, G., Mouvet, C., Albrechtsen, H.-J. Acetochlor sorption and mineralization in limestone subsurface and aquifers, submitted to *Science of the Total Environment*.

IV. Janniche, G., Spliid, H., Albrechtsen, H.-J. Application of microbial Community-Level Physiological Profiles and herbicide mineralization to a herbicide contaminated groundwater, submitted to *FEMS Microbiology Ecology*.

The papers are not included in this www-version, but can be obtained from the Library at DTU Environment. Contact library@env.dtu.dk or Department of Environmental Engineering, Technical University of Denmark, Miljoevej, Building 113, DK-2000 Kgs. Lyngby, Denmark

The Department of Environmental Engineering (DTU Environment) conducts science-based engineering research within four themes:
Water Resource Engineering, Urban Water Engineering,
Residual Resource Engineering and Environmental Chemistry & Microbiology.
Each theme hosts two to five research groups.

The department dates back to 1865, when Ludvig August Colding, the founder of the department, gave the first lecture on sanitary engineering as response to the cholera epidemics in Copenhagen in the late 1800s.

DTU Environment
Department of Environmental Engineering
Technical University of Denmark

Miljoevej, building 113
DK-2800 Kgs. Lyngby
Denmark

Phone: +45 4525 1600
Fax: +45 4593 2850
e-mail: reception@env.dtu.dk
www.env.dtu.dk

ISBN 978-87-91855-90-0